Synthesis of carolacton precursors

Von der Fakultät für Lebenswissenschaften

der Technischen Universität Carolo-Wilhelmina zu Braunschweig

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

(Dr. rer. nat.)

genehmigte

D i s s e r t a t i o n

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aus Guntupalli / Indien

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eingereicht am: 04.08.2014
mündliche Prüfung (Disputation) am: 03.11.2014

Druckjahr 2014

Vorveröffentlichungen der Dissertation

Teilergebnisse aus dieser Arbeit wurden mit Genehmigung der Fakultät für Lebenswissenschaften, vertreten durch den Mentor der Arbeit, in folgenden Beiträgen vorab veröffentlicht:

Publikationen

M. Reck, K. Rutz, B. Kunze, J. Tomasch, S. K. Surapaneni, S. Schulz, I. Wagner-Dobler: The Biofilm Inhibitor Carolacton Disturbs Membrane Integrity and Cell Division of *Streptococcus mutans* through the Serine/Threonine Protein Kinase PknB, *J. Bacteriol.* **2011**, *193*, 5692–5706.

Tagungsbeiträge

Surapaneni, S. K., Wagner-Döbler, I., Schulz, S.: Synthesis towards the carolacton side chain. (Poster) 18. ORCHEM, Tagung der Gesellschaft Deutscher Chemiker (GDCh), Weimar (2012).

I Dedicate this success to my loving wife Shanti Surapaneni, my daughter Saanvi Surapaneni, and to my parents Satyavathi Surapaneni and Venkata Ratnam Surapaneni.

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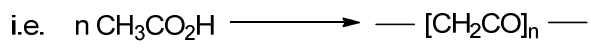
1 Introduction

1.1 Polyketide natural products

The polyketide natural products are a group of secondary metabolites that exhibits remarkable diversity both in terms of their structure and function. They represent a unique class of biologically active natural products.[1] These metabolites are ubiquitous in distribution and have been reported from organisms as diverse as bacteria, fungi, plants, insects, dinoflagellates, mollusks and sponges. In addition they boast a wealth of medicinally important activities including antibiotic, anti-cancer, antifungal, antiparasitic and immunosuppressive properties.[2]

The wide spectrum of activity of polyketides makes them economically, clinically and industrially the most sought after compounds. Their diverse structures can be explained as being derived from poly-β-keto chains, formed by coupling of acetic acid (C_2) units via condensation reactions. Thus *polyketides* are defined as a class of molecules produced through the successive condensation of small carboxylic acids.[3]



The history of subsequent theorising on the biosynthetic pathway to the alkaloids was first led by James Collie, followed by Robinson's and Arthur Birch hypotheses. The boost to the resurgence of polyketides came from Arthur Birch in the 1950s until the innovation of NMR took place. His contributions were crucial for two of the reasons. Firstly, he recognised that polyketones could be generated from acetate units by repetitive condensation reactions and secondly, he was willing to test his theory by supplying an isotopically labeled version of his proposed precursor to a suitable polyketides producing organism. He had chosen the aromatic polyketide 6-methylsalicylic Acid (6-MSA) (**1**) an antibiotic produced by the fungus *Pencillium patulum*, also an intermediate in the synthesis of toxic patulin (**2**).The mechanistic basis of his hypothetical proposal was the four acetate units linked 'head-to-tail' to generate a triketo acid with series of reactions leading finally to the six membered carbocyclic aromatic natural product (Figure 1).[4]

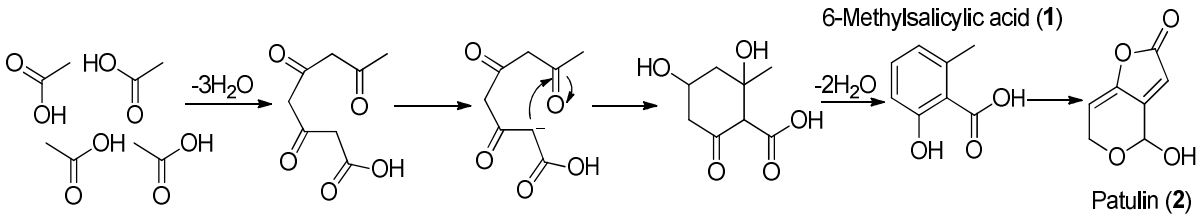


Figure 1: Birch's proposed biosynthetic reaction sequence of 6-Methylsalicylic acid (**1**)

He tested his ideas by feeding acetate labelled with ^{14}C at C-1 (Figure 2). According to his hypothesis four sites in 6-MSA (**1**) should incorporate the radio activity as indicated. It was therefore essential to determine the pattern of labelling in the molecule to confirm his hypothesis. This analysis involved laborious experiments in which samples of acid were subjected to controlled chemical degradation to produce fragments that could be reliably correlated with specific sites in the natural product. The three of these fragments were isolated and subjected to radioactivity measurement to determine their 'relative molar activity' and the results were consistent to his predictions. Feeding of the radiolabeled acetate to many organisms known to produce secondary metabolites yielded structures exhibiting a 'measles pattern' of isotopic spots. These experiments not only stood as a basis that many polyphenolic aromatic molecules were biosynthesized from acetate units according to Collie-Birch polyketide hypothesis but also that many non-aromatic compounds were also formed by further transformations of such products.

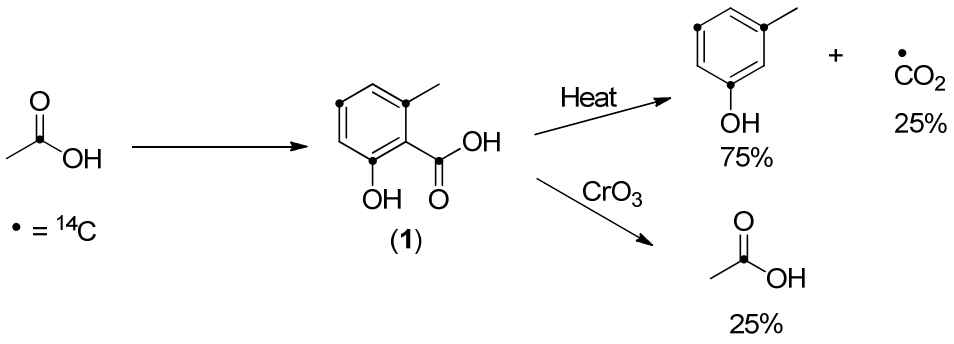


Figure 2: Birch's demonstration of 6-MSA (**1**) derived from four acetate units[4]

Polyketides can be classified into aromatic and complex structural classes. The former are built mainly from acetate units through a reiterative process wherein the β–carbonyl groups formed after each condensation cycle are left largely unreduced.[5] Complex polyketides are composed of acetates, propionates or butyrates and the extent of β–carbonyl reduction varies from one cycle to the next. Some polyketides have more than one pharmacological effect. For instance, the antibiotic erythromycin (**3**)[6] induces gastric contractions and can act as a motilin agonist. Of the polyketides the smallest is 6-methylsalicylic acid (**1**)[4] whereas one of the the largest is brevetoxin B (**4**)[7] with 50 carbon atoms in its chain which are shown

in Figure 3. Many acetate-derived polyketides cyclize to form aromatic rings (e.g. anthracyclines) or lactone rings (e.g. macrolides, poly-enes). The majority of polyketides are produced by the actinomycetes as secondary metabolites.[8] The compounds are subdivided into structural subgroups: macrolides (e.g. spiramycin), polyethers (e.g. monensin), polyenes (e.g. amphothericin), and aromatic compounds (e.g. thysanone).[3]

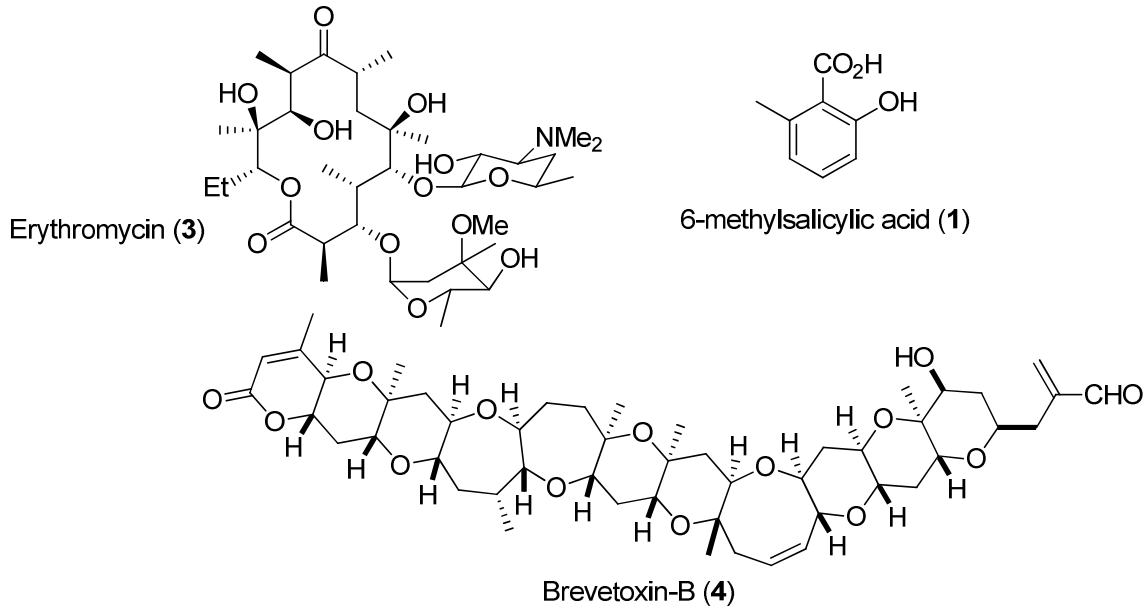


Figure 3: Some examples of polyketides

Polyketides are a specific class of secondary metabolites that are produced by the polyketides synthases (PKSs) which iteratively construct the molecular framework from consecutive condensations of acyl units. Being structurally diverse, all polyketides are assembled by successive rounds of decarboxylative Claisen condensations between a thioesterified malonate derivative and an acyl thioester (Figure 4).[9]

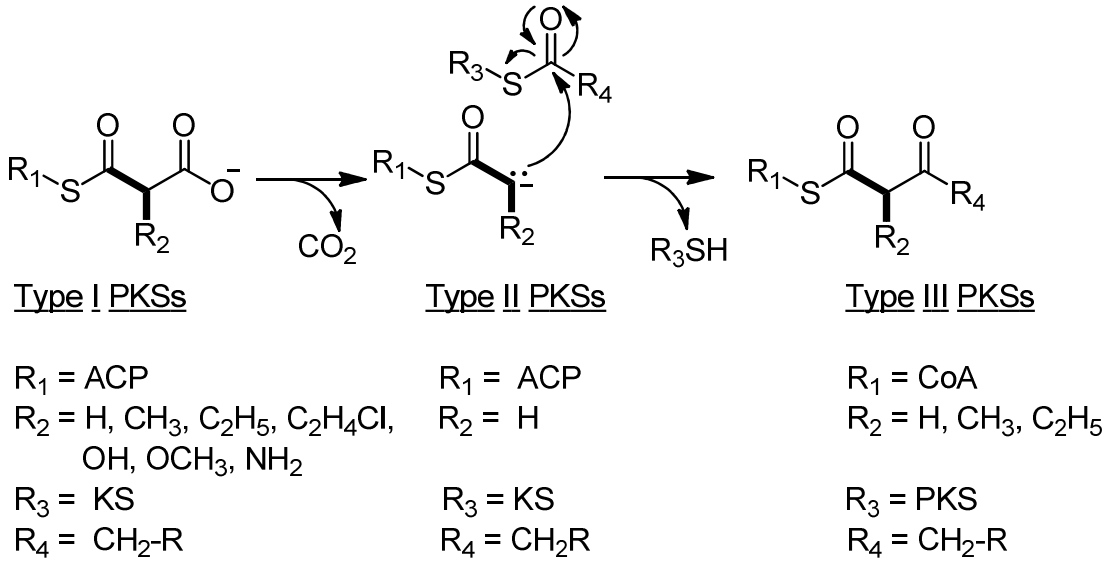


Figure 4: Basic mechanism of decarboxylative Claisen condensations

The enzymes that catalyze these condensations are referred to as polyketide synthases. PKS systems consist of reactive domains that modify a growing molecular structure in a similar fashion to an assembly line. PKSs are large multienzyme protein complexes that contain a coordinated group of active sites. The biosynthesis occurs in a stepwise manner from simple two, three, four carbon building blocks such as acetyl-CoA, propionyl-CoAa, butyryl-CoA and their activated derivatives, malonyl-CoA, methylmalonyl-CoA and ethylmalonyl-CoA. When the substrates are chiral like methylmalonyl-CoA, the corresponding acyltransferases exhibit strict stereospecificity. The α–carboxylated substrates (e.g.; malonyl-CoA and methylmalonyl-CoA) are sources of extender units, whereas neutral substrates such as acetyl-CoA are sources of primer units for polyketides chain synthesis (Figure 5a, 5b).[10]

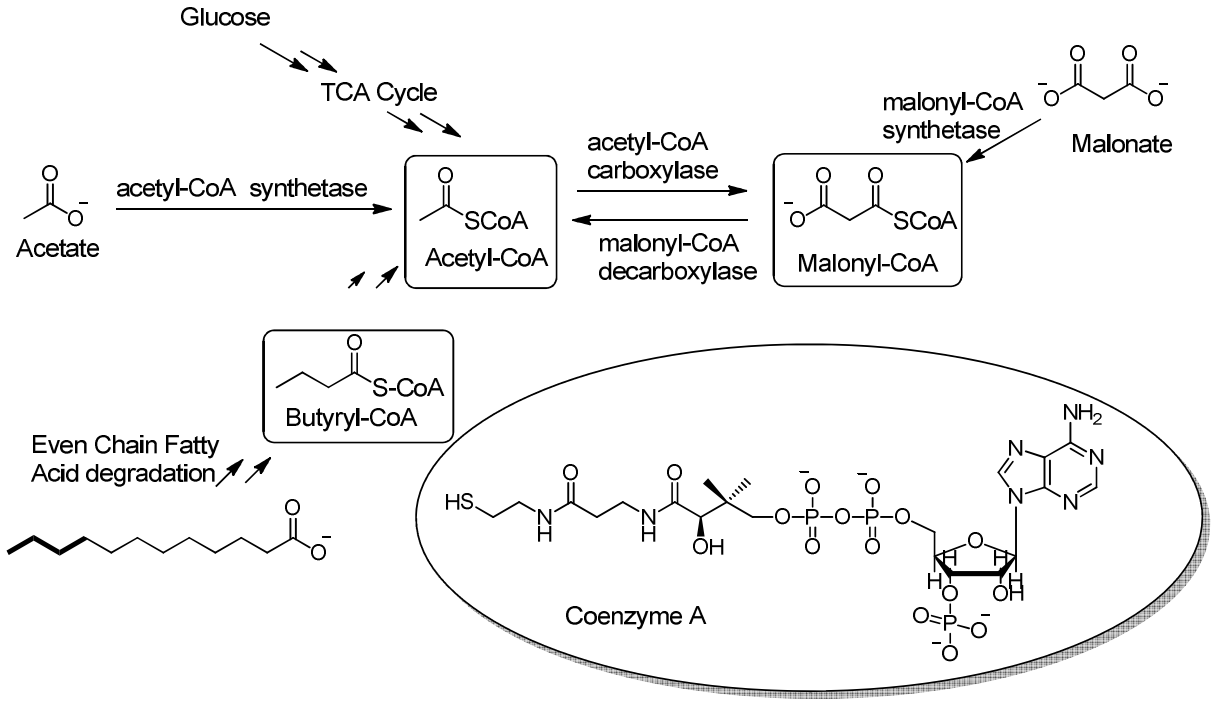


Figure 5a: Polyketide synthase substrate routes having potential substrates boxed

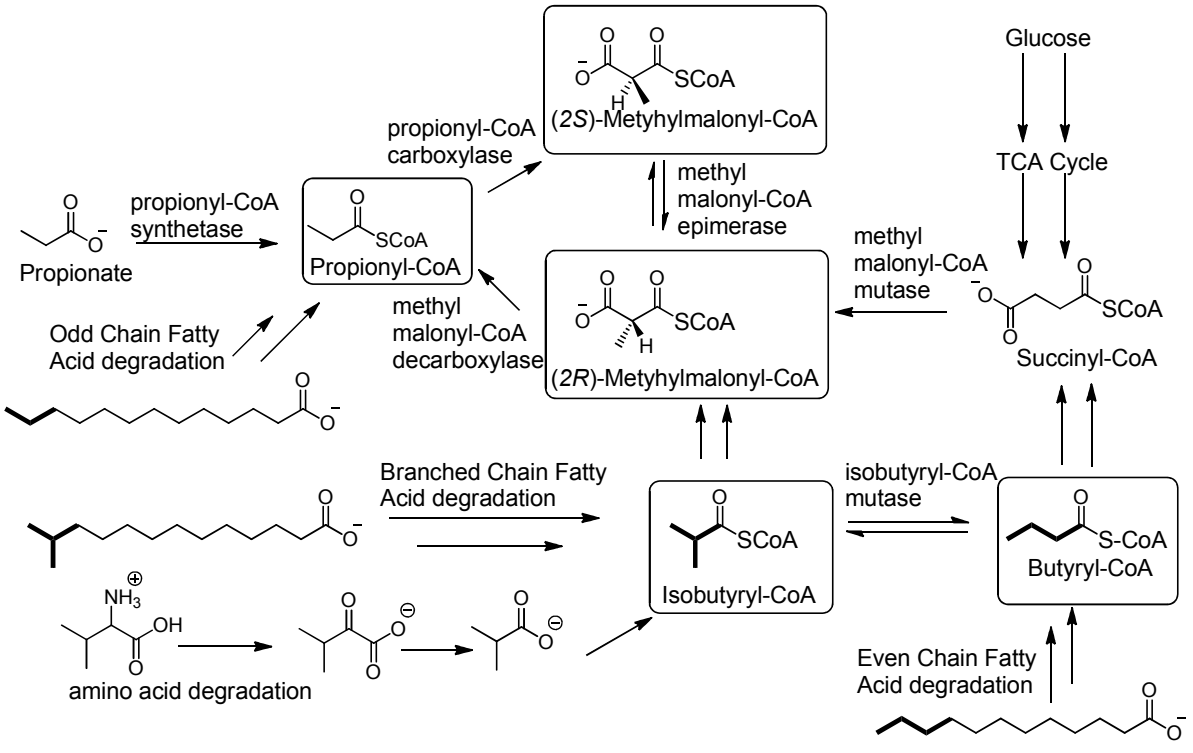


Figure 5b: Polyketide synthase substrate routes having potential substrates boxed

Due to their mechanistic similarities[11,12] (Figure 6) in between the fatty acid biosynthesis and polyketides biosynthesis, PKS are classified using the nomenclature for types of fatty acid synthases with some modifications added according to its needs.

PKSs are classified into three groups.

Type I polyketide synthases

Type II polyketide synthases

Type III polyketides synthases

Type I polyketides synthases are further subdivided into Iterative PKSs and Modular PKSs; whereas Iterative PKSs are still further subdivided into

NR-PKSs – non reducing PKSs (Products: True polyketides)

PR-PKSs – partially reducing PKSs

FR-PKSs – fully reduced PKSs (fatty acid derivatives).

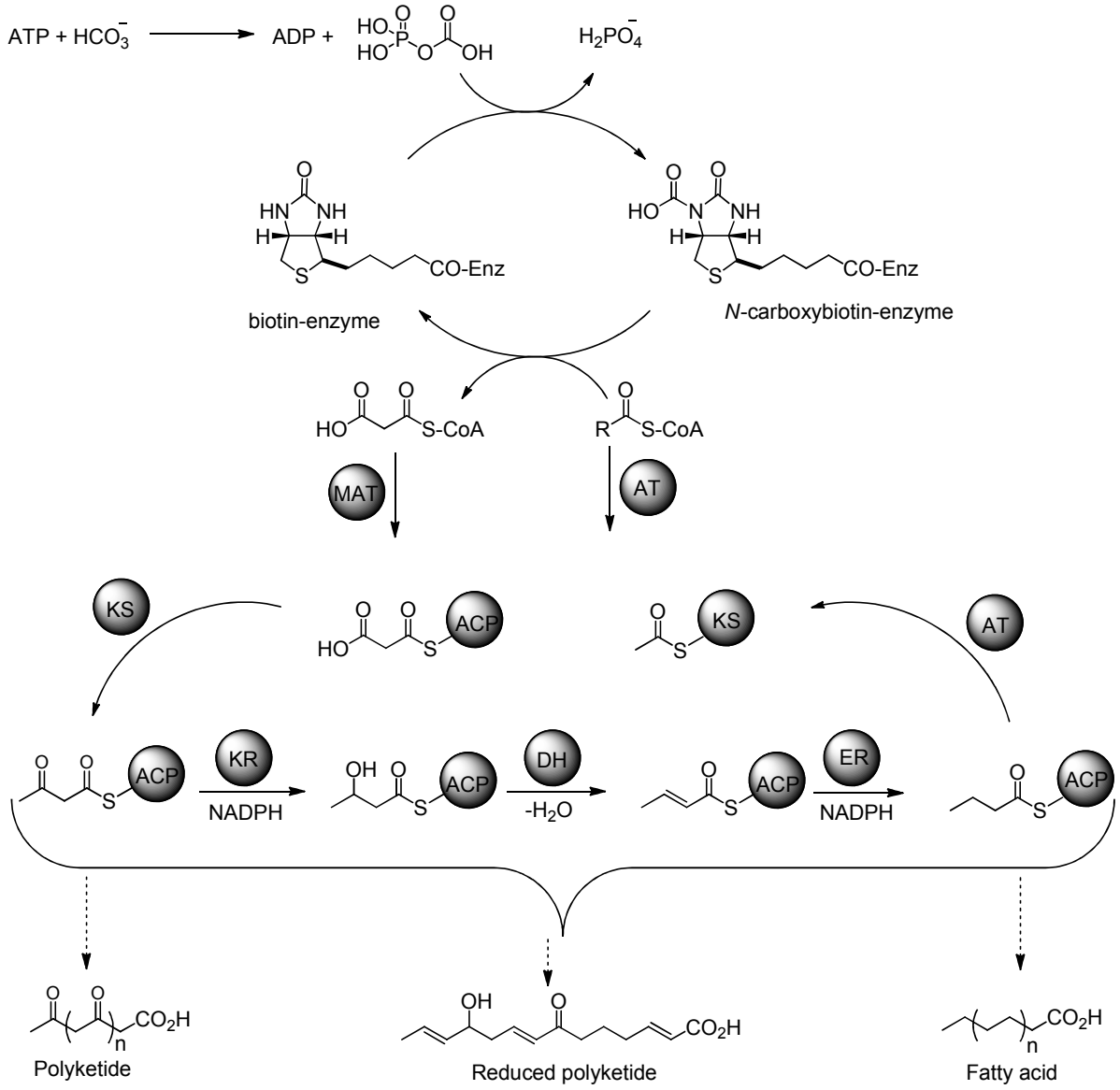


Figure 6: Generic reaction scheme for biosynthesis of both fatty acids and polyketides[11,12]

The modular system is the classical bacterial type I PKS best exemplified by the PKS responsible for assembling 6-deoxyerythronolide B synthase (DEBS) for the biosynthesis of reduced polyketides (e.g. macrolides, polyethers and polyene) such as erythromycin A (**3**) (Figure 7). This PKS assembles seven precursors consisting of one propionyl-CoA starter unit and six methylmalonyl-CoA extender units into 6-DEB. The role played by each of these precursors in polyketide assembly is clearly apparent from the unit names: the starter unit is the initiating precursor for polyketides synthesis, while the extender units elongate the polyketides backbone to completion. A set of catalytic domains, grouped together as a "module," controls the incorporation of each precursor into the polyketides backbone.[12]

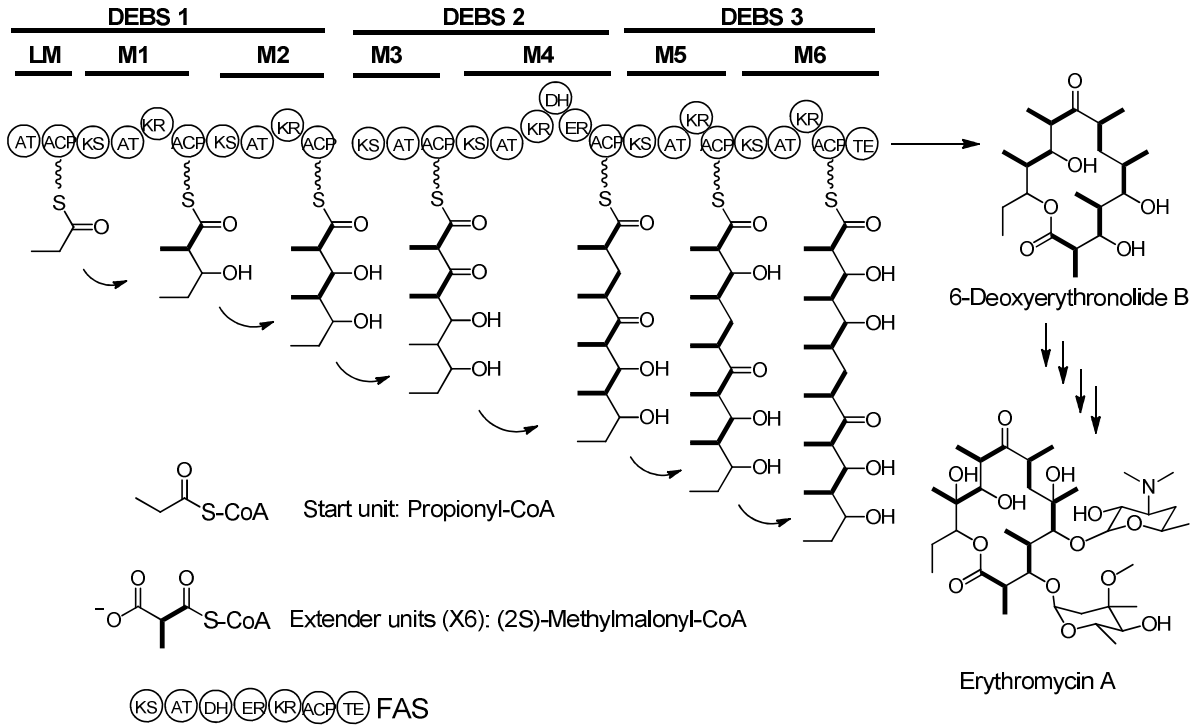
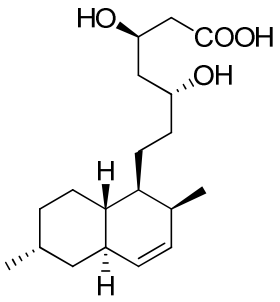


Figure 7: Domain organization of the animal FAS and the DEBS modular PKS[12]

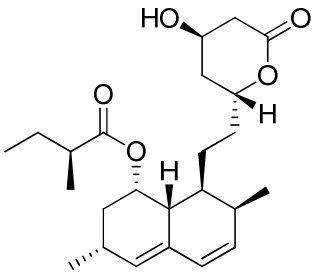
For the modular type I PKSs, the number of modules is equivalent to the number of precursors incorporated into the polyketides. The modules incorporating the starter units can have variable catalytic domains, whereas the modules that incorporate the extender units typically consists of three core domains namely ketosynthase (KS), acyltransferase (AT), and acyl carrier protein (ACP) for polyketides extension and up to three auxiliary domains namely ketoreductase (KR), dehydratase (DH), and enoylreductase (ER) involved in β-keto processing. The AT domain is the "gate keeper" of the module and recognizes the specific extender units that incorporate into the polyketide chain and covalently attached to the malonyl derivative of the extender unit of the thiol group of the ACP domain. The KS domain catalyzes the carboxylative Claisen condensation between a neighbouring ACP-linked malonate derivative and an ACP-linked acyl thioester to extend the polyketide chain. The optional domains KR, DH, ER alter the oxidation state of the β-keto group formed after KS-catalyzed condensations whereas the TE (thioesterase) domain is the termination or releasing module that hydrolyzes the completed polyketides chain from the ACP domain of the previous module.[9]

Iterative type I PKSs utilize the same core catalytic domains as modular type I PKSs, but these domains occur in a single polypeptide that is used repetitively to generate the entire

polyketide backbone. Initially it was thought to be limited to fungal systems, but Iterative type I PKSs were also known to be found to be in many bacteria (e.g. dihydromonacolin L (**5**) an intermediate of lovastatin (**6**) Figure 8).[13]



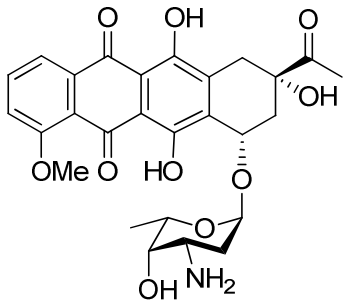
dihydromonacolin L (**5**)



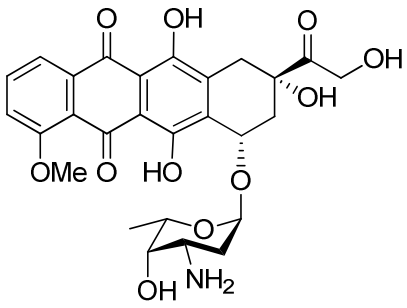
Lovastatin (**6**)

Figure 8: Structure of using Iterative type I PKS system for biosynthesis

Type II PKSs contain similar core catalytic domains seen in type I PKSs, with the exception of having typically two KS domains, KS_α and KS_β. The former is equivalent to the KS seen in the type I PKSs, while the later controls the length of the polyketide and the enzymatic activities that are typically present on individual proteins. The reductive processing of the β-keto groups occurs only after the polyketide is fully synthesized (e.g. two anti cancer drugs daunorubicin (**7**) and doxorubicin (**8**) Figure 9).[14]



daunorubicin (**7**)



doxorubicin (**8**)

Figure 9: Structure of using Iterative type II PKS system for biosynthesis

Type III PKSs, similar to above type I and II PKSs condense a starter unit with a series of extender units to generate a poly-β-keto chain. However differs from the above type I and II PKSs by typically lacking multiple catalytic domains and utilize an ACP-independent mechanism[9] starting from the *p*-coumaroyl-CoA and acts directly on the acyl-CoA substrates (e.g. flavanone (**9a)** and resveratrol (**9b**) Figure 10).[15]

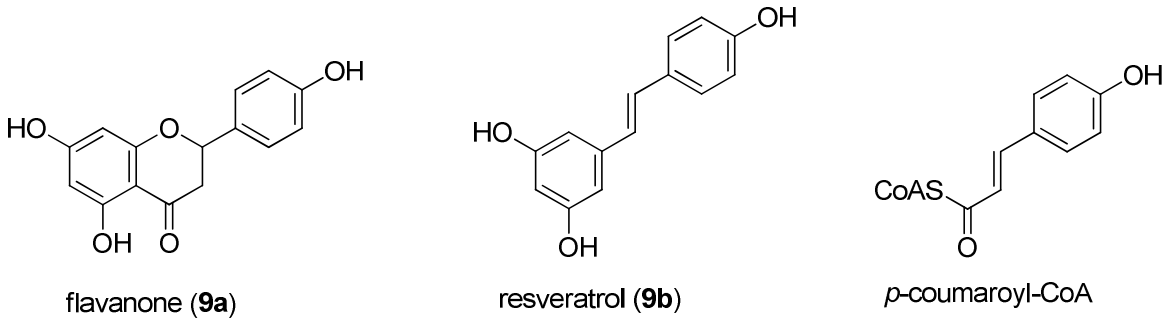


Figure 10: Structures of type III PKS system for biosynthesis and starter unit

Despite structural and mechanistic differences all types of PKSs biosynthesize polyketides by sequential decarboxylative Claisen condensation of the acyl-CoA precursors, and the ketoacyl synthase (KS) domain or subunit catalyzes the C-C bond-forming step.

1.2 Myxobacteria

Myxobacteria are obligate, aerobic Gram-negative mesophilic δ-proteobacteria, which are commonly isolated from soil, the bark of trees, decaying plant material, herbivore dung and the marine environment.[16,17] The discovery of novel myxobacterial species from moderately halophilic soil as well as marine environments, illustrates that they can adapt to a wider variety of environment conditions.[18] All known myxobacteria are united in the order *Myxococcales*, which can be further divided into the three suborders *Cystobacterineae*, *Sorangiineae* and *Nanocystineae*.[19] More than 7500 strains within the order *Myxococcales* have been already isolated by research groups at the Helmholtz centre for Infection Research (Braunschweig, Germany; formerly German Research Centre of Biotechnology (GBF)) and novel strains, species and even families are continually being discovered.[16,20]

These fascinating microbes display several behavioural features that distinguish them from many other bacteria. For example, they move about on solid surfaces by gliding or creeping, in a similar way like how amoebas do.[21] They secrete exo-enzymes which allow them to use a range of biological macromolecules (e.g. cellulose) as food sources, but are also able to prey actively on whole microorganisms such as fungi and bacteria.[22] In addition to these, under starvation conditions, they implement a cooperative developmental program involving

hundreds of thousands of cells.[23] The cells aggregate to form a pseudoplasmodium-like slimy mass, which ultimately transforms into a complex, multi cellular fruiting body (as shown in the Figure 11) harbouring propagative spores. Another notable characteristic of the myxobacteria is their rich secondary metabolism, which places them among the best known natural product producers (*i.e.,* actinomycetes, *Bacillus* species, pseudomonads, and fungi).[24] Furthermore, the genome of myxobacteria is one of the largest known from any bacterium having for e.g. 13.0 MBp for *Sorangium cellulosum* So ce56.[25]

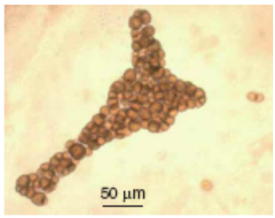


Figure 11: Fruiting bodies formation by *Sorangium cellulosum* (Sorangiineae).[26]

1.2.1 Polyketides from Myxobacteria

Myxobacteria are a particularly rich source of novel polyketides.[27] As mentioned above, in the last three decades, due to the trailblazer work of the groups of prof. Höfle and Prof. Reichenbach at the Helmholtz centre for infection research, an impressive number of structurally unique and biosynthetically diverse polyketides have been reported from these soil-living organisms. In total, they extend to a range of approximately 60 structurally new classes of polyketides and many structural variants.[28] Many of these compounds are associated with biological activities, including antiproliferative, antibiotic, antifungal or antiplasmodial activities. On a molecular level, a wide variety of molecular targets are specifically addressed, including the cytoskeleton, nucleic acid polymerases, respiratory chain, nuclear transport, microfilaments and protein or fatty acid synthesis. This demonstrates that these compounds are highly attractive targets for further development.

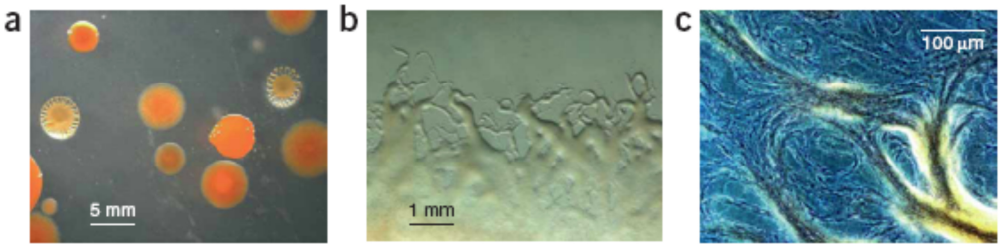


Figure 12: *Sorangium celluslosum* a) colonies and (b, c) swamming growth patterns.[26]

Around hundred distinct core structures and multiples of derivatives are yielded from these above mentioned 7500 approximately identified myxobacterial strains.[29] The majority of these include polyketides, non-ribosomal polypeptides and their hybrids[24,30] whereas other structural types include terpenoids, phenyl_propanoids, and alkaloids. During the past 15 years 15 constitutionally novel polyketides were reported from myxobacteria. The first myxobacerial natural product applied for clinical use was a semi synthetic epothilone B (**10**) derivative named Ixabepilone approved by FDA and marketed in the United States by Bristol-Myers Squibb (BMS) for chemotherapy against breast cancer in 2007 under the trade name Ixempra®.[31] In addition to epothilone the natural products from myxobacteria which interact with cytoskeleton are disorazol (**11**)[32] and rhizopodin (**12**)[33]. All these 3 compounds are potential candidates in cancer treatment. Some 29% of myxobacterial metabolites exhibit anti-bacterial activity.[34] Notably; *Sorangium cellulosum* (Figure 12) was the most productive myxobacterium with caroalcton (**13**),[35] etnangien (**14**),[36] and maracin(**15**) /maracen (**16**)[37] isolated from the various strains of this microorganism which are shown in the Figure 13.

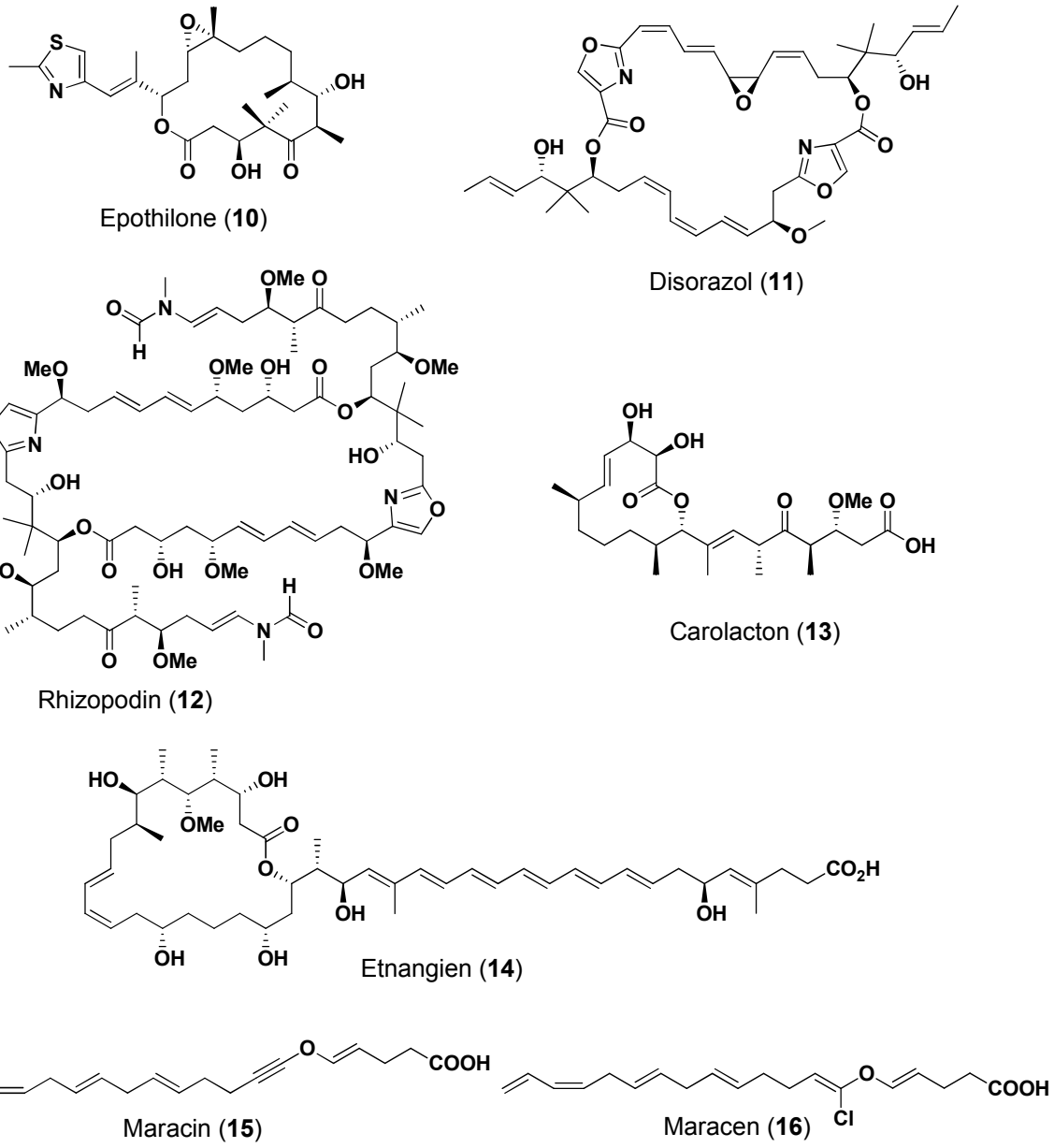


Figure 13: A selection of polyketides from myxobacteria.

1.3 Carolacton

1.3.1 Biofilms and biological activity of carolacton

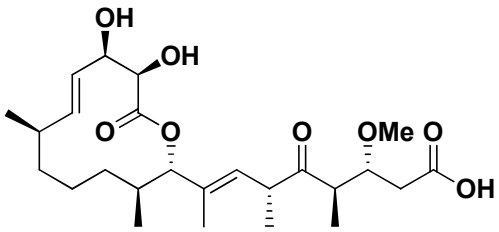
Up to 60% of all human infections are caused by biofilms.[38] Biofilm is a slimy layer formed by bacteria adhering to a implanted medical device or damaged tissues which encase themselves in a hydrated matrix of polysaccharide and protein. Periodontitis, endocarditis and chronic lung infection in cystic fibrosis patients are well known examples of diseases that are generally associated with biofilms.[39] Additionally caries is also a disease that is

associated with biofilms. Bacterial biofilms often conceal from host defences and are inherently resistant to antimicrobial agents. These characteristics result directly in severe health treats. The eradication of biofilms became a major concern in clinical treatment. There is an urgent need to develop new methods or improved tools to ideally and selectively destroy clinically relevant biofilms as the antibiotic resistance of bacteria in biofilms is found to be approximately 1000 folds higher than in plankton form. Biofilm formation and quorum sensing are the central and often interconnected features of bacterial social life. The major classes of theses bacterial social behaviour, and the suggestion that quorum sensing enables bacteria to turn on and off the secretion of extracellular polymeric substances (EPS) so as to increase their competitive ability against other species and strains within biofilms was well addressed by Foster *et al*.[40] Quorum Sensing is defined as a process that many species which inhabit dense, surface-bound communities, termed *biofilms,* within which they communicate *via* signalling molecules and respond to local cell density. Quorum sensing systems might be promising targets in treating biofilm induced infections. Hence the development of anti quorum sensing drugs that specifically disable the virulence might represent an alternative to conventional antibiotic therapy, mainly considering the fact that these antibacterial compounds are less likely to induce the development of resistant bacteria.[41]

Dental caries (Figure 14) and Periodontitis are caused by biofilms known as dental plaque that result from microbial colonization of the tooth surface or the subgingival margin. As said above novel strategies are necessary for battling the clinically relevant biofilms, especially based on the fact that two-thirds of human bacterial infections are caused by biofilm forming bacteria. Under these circumstances our cooperation partner Prof. Dr. Wagner-Döbler and co-workers worked on finding new inhibitors of biofilm formation of Gram positive facultative anaerobic bacterium *Streptococcus mutans*. It is known that more than 500 bacterial species are found in dental plaque. Out of these *Streptococcus mutans* is considered to be the principal pathogen responsible for the caries disease in human oral cavity.[41] During the metabolism of dietary carbohydrates *Streptococcus mutans* rapidly produce acid end products lowering the pH to approximately pH 3.5 resulting in demineralisation of dental enamel and caries formation.

Figure 14: Caries cavities (arrows) left and right buccal view.[42]

In this project Prof. Dr. Wagner-Döbler et al. tested several secondary metabolites from *Sorangium cellulosum* on their effectiveness in the inhibition against biofilm formation which have shown no antifungal and antibiotic activity in previous studies. They found the secondary metabolite carolacton (Figure 15), produced by the *Sorangium cellulosum*, inhibits biofilm formation of *Streptococcus mutans*.[41] Figure 15 shows the structure of carolacton which was elucidated by Prof. Dr. Andreas Kirschning et al.[35] Carolacton induced the damage of *Streptococcus mutans* biofilms at nanomolar concentrations. Carolacton **(13)** is a myxobacterial secondary metabolite isolated in 1998 from *Sorangium cellulosum*, strain So ce960 by Höfle et.al. Carolacton proved to possess high activity against biofilm formation. Thereby Prof. Dr. Wagner-Döbler started the BIOINSYS project towards the studies of carolacton for further studies. This chapter deals with the discussion of the work of the Prof. Dr. Wagner-Döbler et al. recently published.[41]



Carolacton (**13**)

Figure 15: Structure of carolacton[35]

The effect of carolacton on planktonic culture and biofilms tested using LIVE/DEAD staining is shown in Figure 16. The results prove that it does not have any effect on the cells of planktonic culture of *S. mutans*, however, a considerable effect was observed on biofilms. The biofilms were cultivated under anaerobic conditions (80% N_2, 10% H_2, 10% CO_2) on microtitre plates for 24 h at 37 °C. Attempts were made to quantify the extent of biofilm damage caused by carolacton by determining colony forming units (CFU), which was done by using the LIVE/DEAD *BacLight* bacterial viability staining as it was more sensitive and the fastest method. LIVE/DEAD *BacLight* bacterial viability staining kit consists of two stains, propidium iodide and SYTO9, which both stain nucleic acids. When used alone green fluorescing SYTO9 generally labels all bacteria in a population, whereas red fluorescing propidium iodide only penentrates bacteria with damaged membranes, causing a reduction in the SYTO9 stain fluorescence. Thus with an appropriate mixture of the SYTO9 and propidium iodide stains, bacteria with intact membrane stain green and bacteria with damaged membranes stain fluorescent red. Biofilm damage was calculated as the ratio of green versus red fluorescence of the biofilm cells normalized against the untreated control.

This shows the membrane integrity of the cells, as the red fluorescing dye can only enter cells with the damaged membranes.

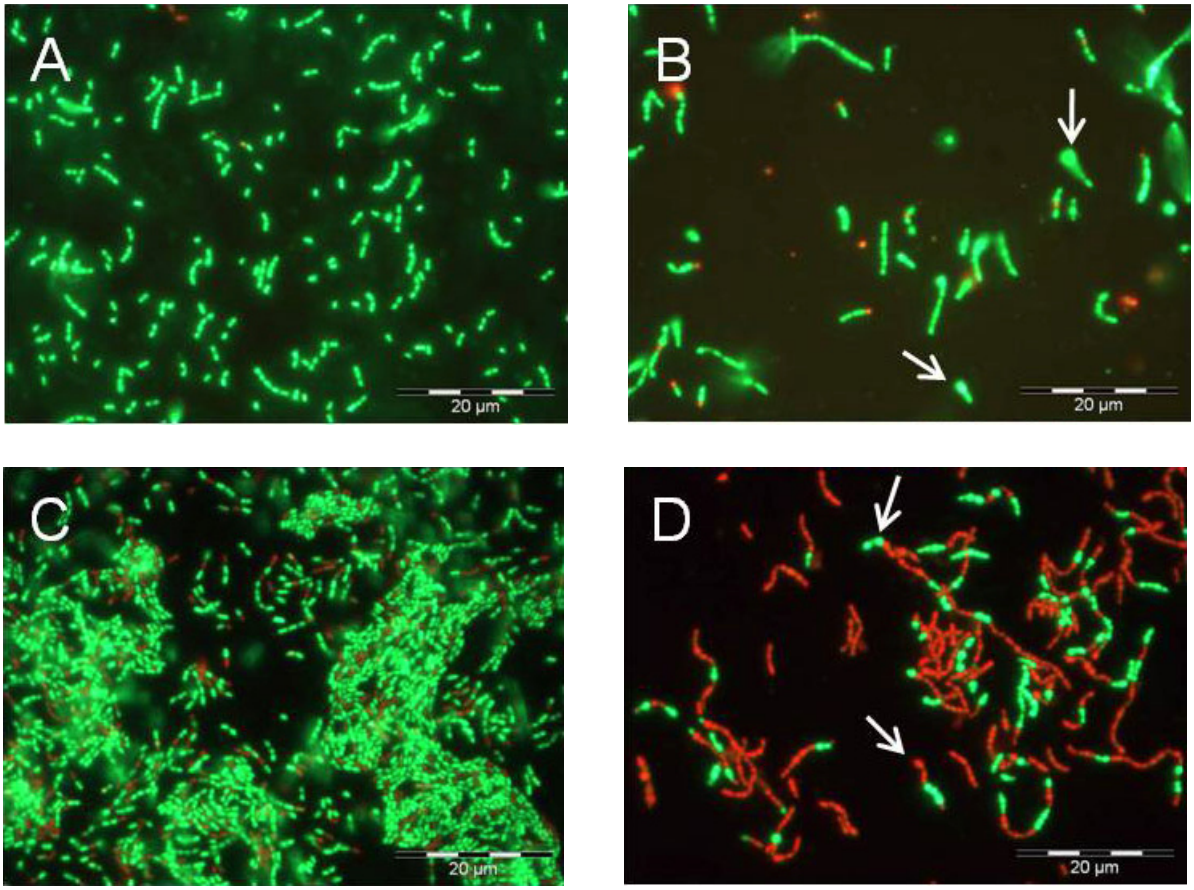


Figure 16: In the above shown Fig A, B are fluorescent contrast images of planktonically grown cultures and C, D are biofilm cells of *Streptococcus mutans*. After LIVE/DEAD staining A, C are without carolacton and B, D are with 5.3 µM carolacton.[41]

The above figure 16 clearly illustrates that the majority of the biofilm cells of *S. mutans* grown anaerobically in the presence of 5.3 µM carolacton showed red fluorescence in Figure 16 D, indicating damaged membranes and possibly death of cells, while planktonic cells are fluorescing green like in the untreated controls in Figure 16 B. Moreover changes in cell morphology were observed, both in planctonic culture and in biofilms. In carolacton treated planktonic cultures in Figure 16 B cells appeared elongated, tended to form longer chains and some cells formed bulges, both as individuals and when growing in chains, suggests that cell division or acid tolerance could be influenced by carolacton. Nearly all of the planktonic cells in Figure 16 A and B were stained green, including also most of the balloon-like ones, which indicates that these cells are indeed viable. In addition, carolacton treated biofilm cells in Figure 16 D show similar morphological modifications, yet many of the cells including also most of the balloon-like ones, are stained red which is easier to distinguish

compared with Figure 16 B which is untreated biofilm. Thus it's evident that membrane damage resulting from carolacton treatment appears to be specific for biofilm cells.[41]

All together, it was shown that there is no significant effect of carolacton on actively growing cells at a pH of above 5.8. These results were concluded by observing the Figure 17 which shows the amount of biofilm cells determined by crystal violet staining.

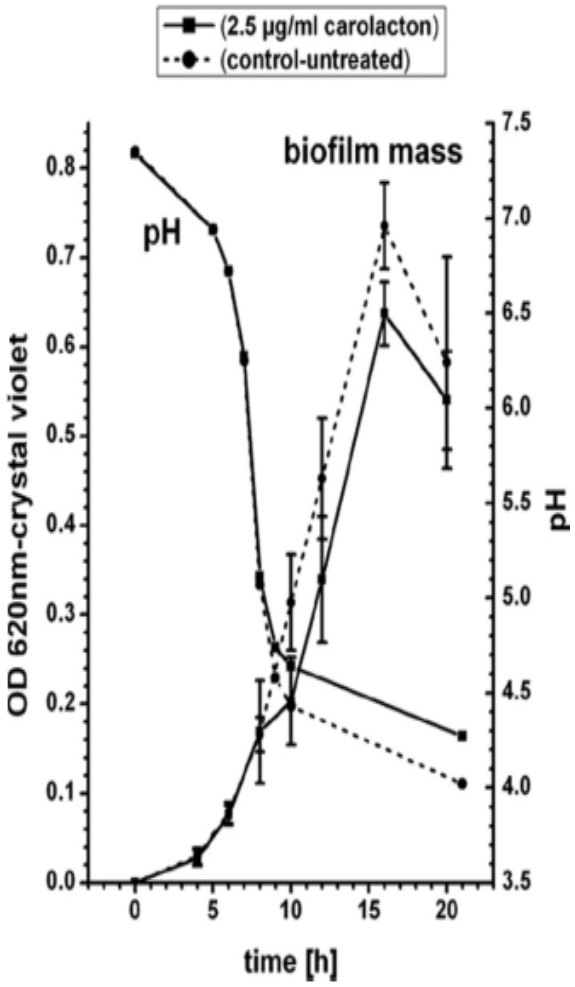


Figure 17: Effect of Carolacton on biofilm mass and corresponding pH.[43]

From the (Figure 17) it is clearly evident that there is no difference between treated and untreated cells for the first 8 h. Between 8 h and 10 h the carolacton treated biofilms show a slightly reduced amount of cell mass compared to the controls. This difference could not be compensated during the remaining biofilm cultivation, leading to a slightly reduced amount of cell mass in the carolacton treated biofilms after 22 h of growth. Carolacton added to the buffers which are not able to grow caused no inhibition of biofilm viability, indicating that the growth of the biofilm is necessary for the inhibitory effect caused by carolacton. Therefore, it can be conclude at this point that there is no significant effect of carolacton on actively growing cells at a pH of above 5.8.

To demonstrate the influence of the growth rate on the activity of carolacton, the inhibitor was added to the medium at different growth phases of the planktonic cultures growing at an acidic pH.[43]

Carolacton was supplemented at the lag phase (condition 18 A), at the early exponential phase (condition 18 B), at mid-exponential phase (condition 18 C), at the onset of the stationary phase (condition 18 D) and at the stationary phase (condition 18 E).The above all cultures are incubated for another 20 h and inhibition of viability was determined by LIVE/DEAD staining illustrated in Figure 18.

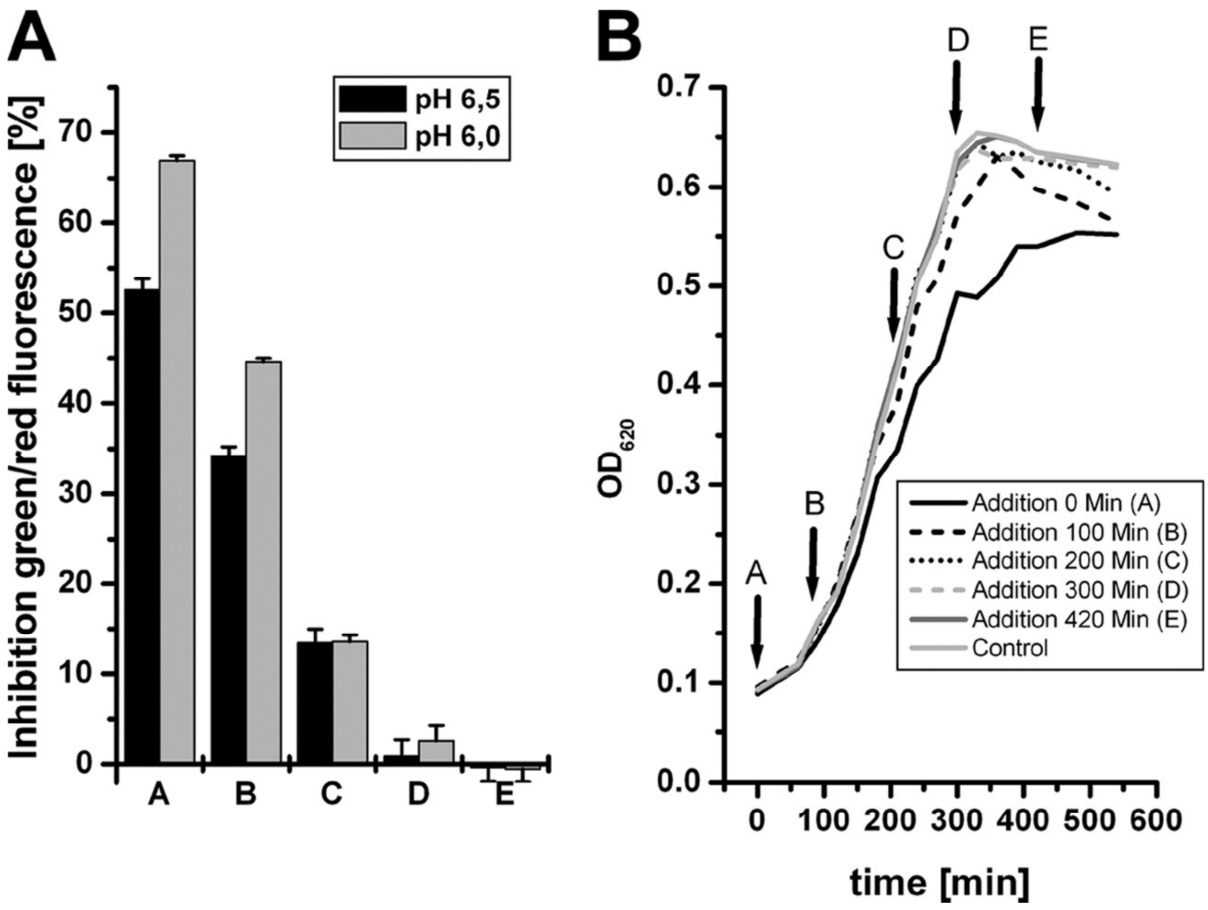


Figure 18: Role of growth phase in the membrane damaged caused by carolacton in palnktonic culture.[43]

A: Inhibition of viability determined by LIVE/DEAD viability staining
B: Growth of planktonic cells at pH 6

Addition of carolacton to proliferating cells (conditions A, B and C) shows inhibition of viability, while stationary phase cells (conditions D and E) are not inhibited. It illustrates that the damage caused by carolacton was increased if the initial pH was lower, but only for the early stages of culture (conditions A and B). The data from Figure 18 show that there is no significant difference in the activity of carolacton between planktonic and biofilms cells indicating that both are affected while they are growing at low pH.[43]

Furthermore the effluence of a large mass consisting, of DNA and β-galactosidase enzyme into the supernatant of carolacton treated biofilms was identified which demonstrates the formation of the large holes in the bacterial membrane due to the sterically complex large molecules. Carolacton caused leakage of cytoplasmic content but no complete cell lysis. It is inferred that carolacton disturbs a process which coordinates cell wall metabolism rather than directly inhibiting a certain enzymatic step in the biogenesis of the cell wall. This process appears to be only during the cell wall formation, because the addition of carolacton after the growth of the cells did not show any inhibitory effect. This mode of action could be a new strategy for the effective antibacterial agents. Existing drugs target the disruption of the synthesis of DNA and proteins and are therefore active only on growing bacteria. This is also the case for carolacton but still it could be a promising application by being an active ingredient in the destruction of cell membrane.[43]

1.4 Published carolacton syntheses

Since the date of patenting[44] the invention of the carolacton and its derivatives in 2009 by Wagner Döbler et al., followed by the structural elucidation in 2010 by Kirschning et al.,[35] the syntheses of carolacton was initiated. The first total synthesis[45] reported till date was by A. Kirschning et al. in 2012 and a partial synthesis, the precursor of carolacton was carried out by Yadav et al.[46]. Recently there was a longest linear sequence (14 steps) synthesis of carolacton reported by Phillips et al.[47]

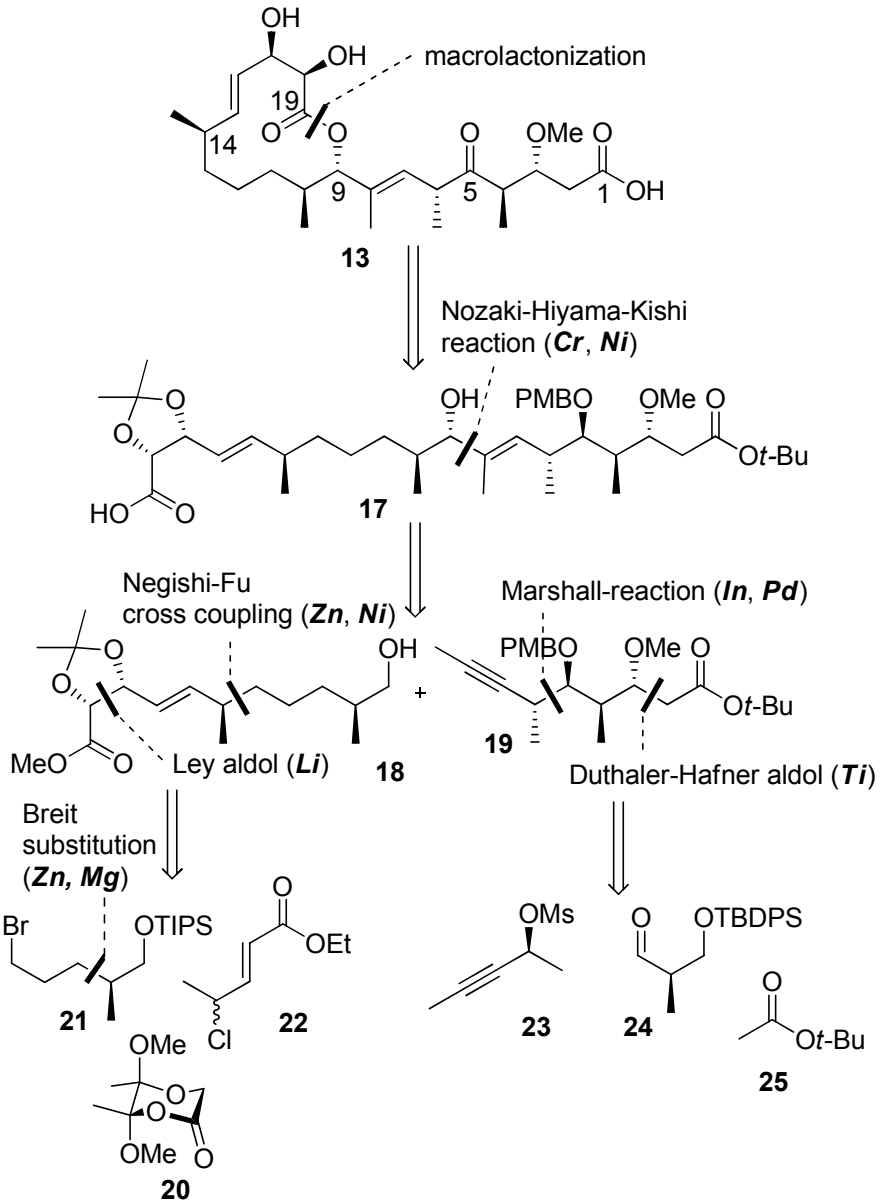


Figure 19: Retrosynthetic scheme of carolacton (**13**) by the kirschning et al.[45]

Kirschning et al. synthesized carolacton through a metal mediated C-C coupling reaction (Figure 19). The synthesis of carolacton was consummated by the macrolactonization of the

crucial intermediate seco acid **17**. The advanced intermediate seco acid was divided into western and eastern fragments. The western fragment prepared by the building blocks **20** to **22** by an aldol reaction according to Ley and an asymmetric Negishi reaction between the bromide and allyl chloride. The eastern fragment synthesized by series of two reactions; asymmetric Marshall reaction between propargylic mesylate and aldehyde obtained from the starting materials 1,2 dibromopropane and 3-hydroxy-2-methylpropionate respectively. The product then upon Duthaler-Hafner Aldol reaction with titanium enolate obtained from *tert*-butyl acetate resulted in the eastern fragment.[45]

Recently reported synthesis by Phillips et al. includes the longest linear sequence of reactions (Figure 20). The C12-C19 segment of the molecule was obtained from the starting material Gulono-lactone derived diol and the C1-C11 fragment including the side chain was initiated with the β-ketoimide using Evans auxiliary and Paterson aldol conditions. The two fragments acid and alcohol are coupled by Steglich-esterification to obtain the ester followed by the ring closing metathesis reaction (RCM) to obtain the lactone with the protecting groups. Later a series of reactions involving crucial selective hydrogenation, cleavage of the protecting groups followed by oxidation and removal of the acetonide group led to the carolacton.[47]

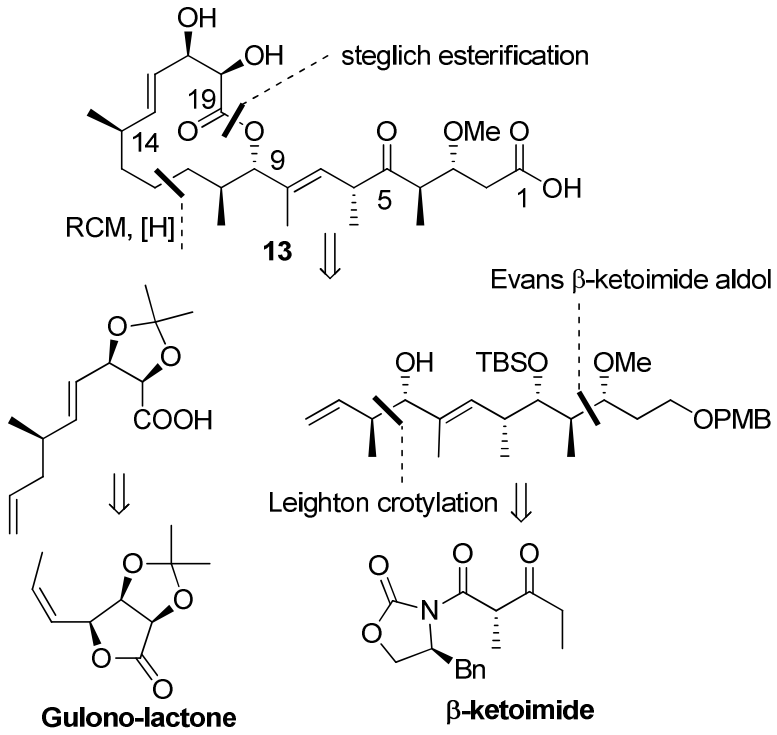


Figure 20: Retrosynthesis scheme of carolacton (**13**) by Phillips et al.[47]

The other partial syntheses as shown in Figure 21 by Yadav et al.[46] in 2012 was initiated with (-)-β-citronellene and D-Ribose as starting materials and Nozaki Hiyama Kishi reaction to prepare the intermediate and were in due to complete the total synthesis involving ring closing metathesis under Yamaguchi macrolactonization conditions.

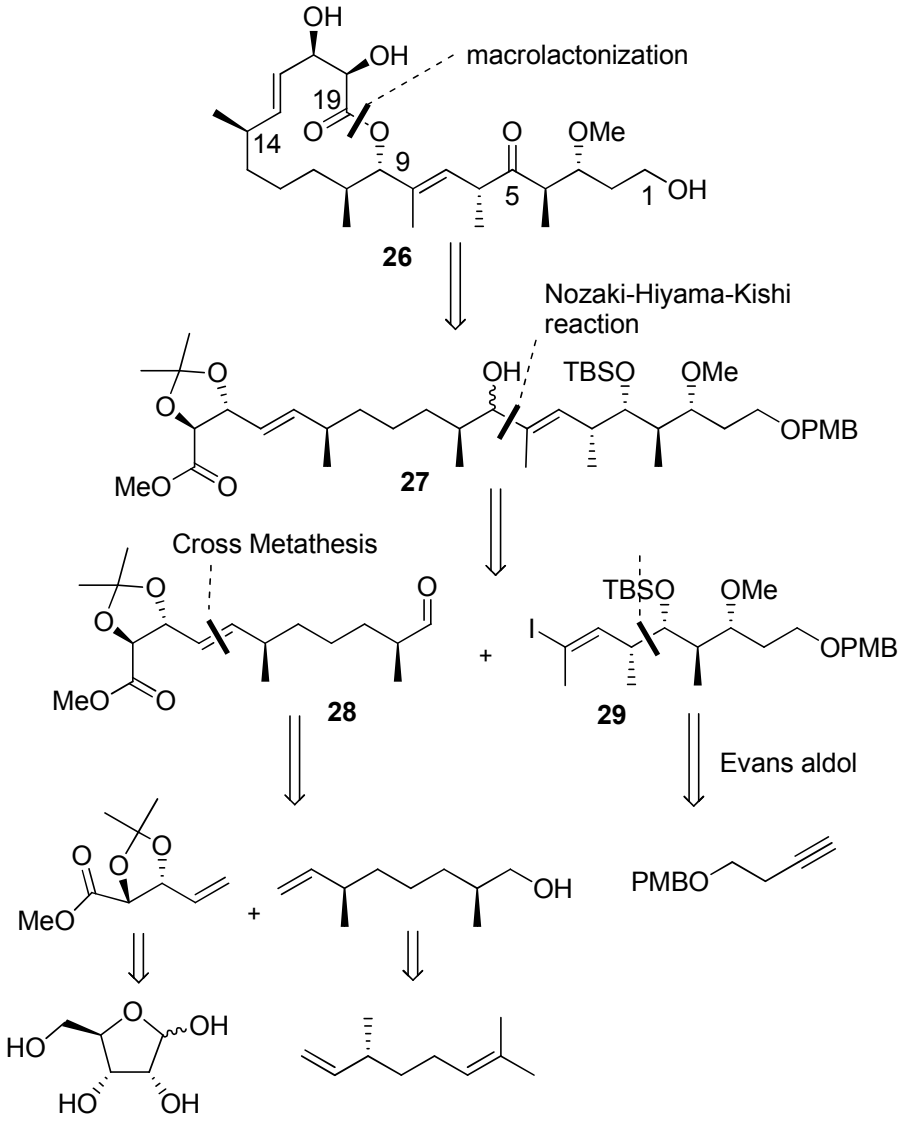


Figure 21: Retrosynthesis scheme of carolacton (**13**) by Yadav et al.[46]

2 Aims of the thesis

As described in the chapter 1 of this work, the synthesis of carolacton and its derivatives is of immense importance because the biological activity tests described defines for continuous access to sufficient quantities of substance. This goal was an important part of a project funded by the Federal Ministry of Education and Research in Germany under the name BioInSys "Medical systems Biology- MedSys" program.

BioInSys project was a large joint project consisting of several working groups which were shown below in the schematic chart (Figure 22).

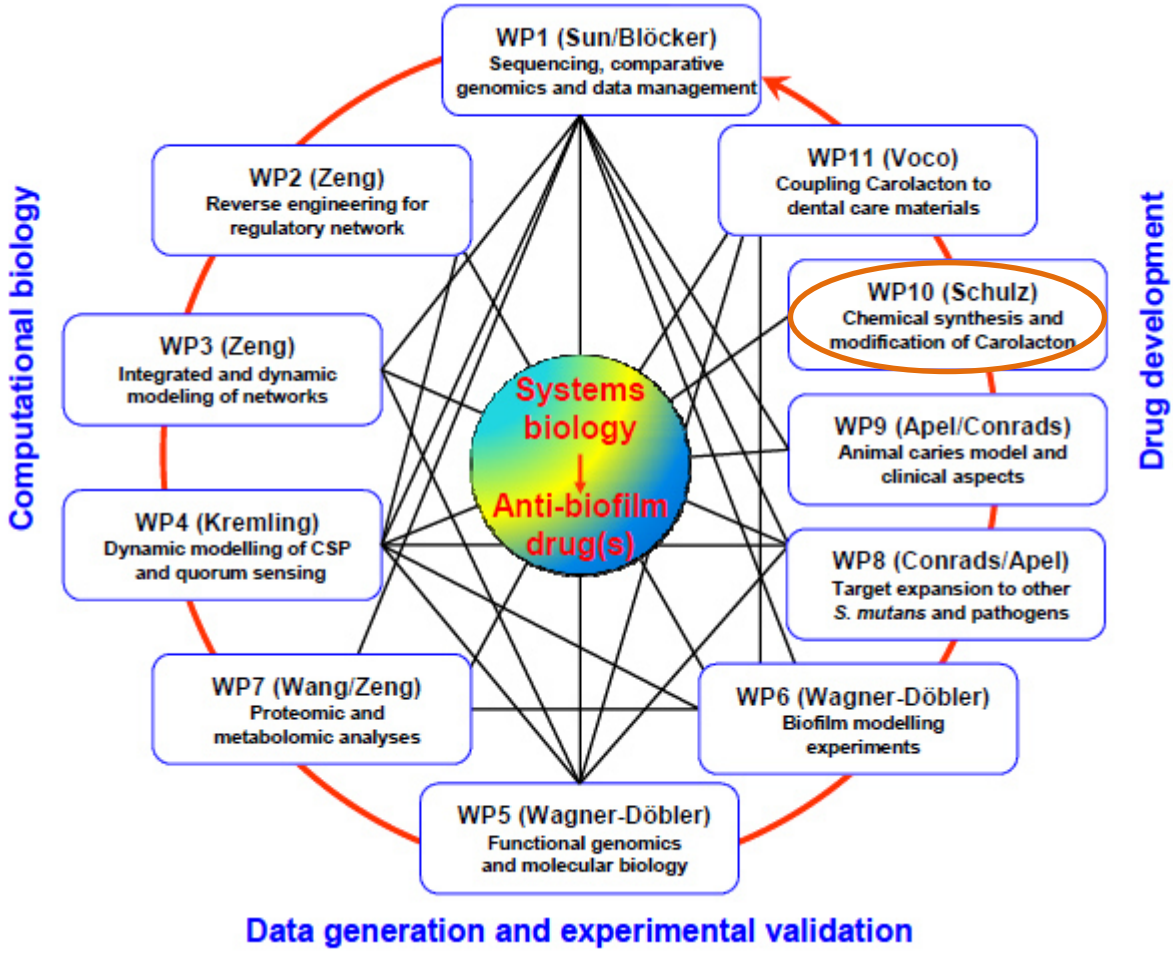


Figure 22: Working groups in the project BioInSys (WP = work package).

The work group of Prof Dr. An-Ping Zeng from the University of Hamburg works on the proteomics and metabolic analysis.[48] The changes in the metabolic, regulatory and

signalling networks can be made by having thorough knowledge on the metabolism and its modules as well as their regulation combined with computer stimulations and quantitative analysis of the metabolites. The research group of Prof. Dr. Andreas Kremling from Technical University of Munich deals with the systems Biology and dynamic models. Here we can understand the cellular behaviour effect by inclusion of the proteomic and metabolic analysis. The research of the workgroup of Prof. Dr. Wagner-Döbler from HZI (Helmholtz centre for Infections Research) is discussed in chapter **1.3**. In order to test caries inhibiting effect not only on biofilms in vitro but also on the clinical trials (animal models), carolacton was applied to the teeth of mouse by mixing it in the drinking water carried out by the workgroups of Prof. Dr. Georg Conrads and Prof. Dr. Christian Apel in the University Clinic of RWTH Achen. The effect of carolacton on the dental materials was tested by the dental company Voco Gmbh. Dr. Andree Barg deals with the composition of the coatings and fillings, which are added to carolacton to be subsequently introduced into human tooth.

The aim of the project is the stereoselective synthesis of the side chain of carolacton and its derivatives. The synthesis of the methyl ester derivative of the carolacton and its role played by it in determining the biological activity of carolacton is also discussed. In addition to it the stability of the carolacton in different buffer solutions is determined using LC/MS.

In this thesis, scheme for the synthesis should be developed to synthesize the carolacton side chain in a simple and stereoselective manner making it feasible to alter the functional groups in order to prepare derivatives of carolacton. To make it accessible to synthesize various derivatives of carolacton, carolacton was divided into three parts as shown in the Figure 23. Fragment 3 is the part of this wok whereas the synthesis of Fragments 1 and 2 were carried by Insa Bergmann and are not reported here.

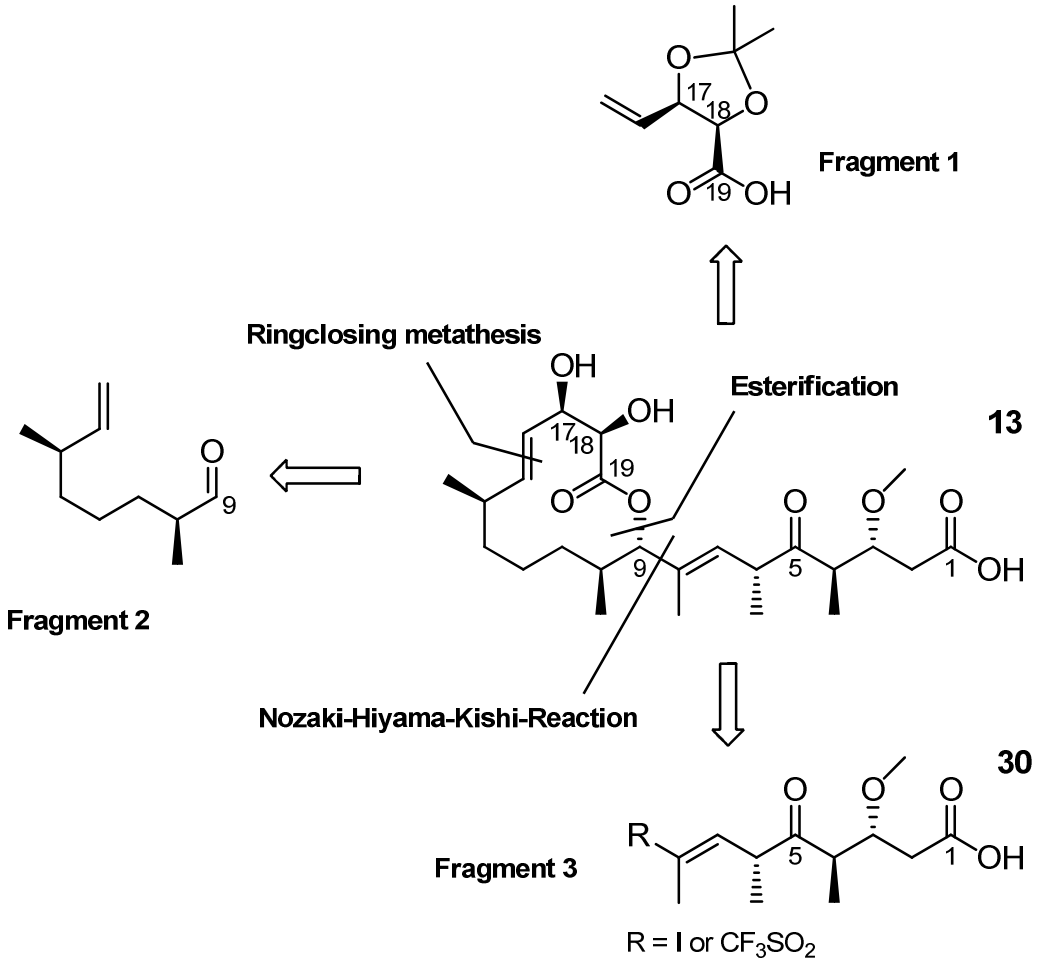


Figure 23: Retrosynthesis of carolacton.

In the above retro synthesis the macrolide ring is synthesized from fragments 1 and 2. Fragment 1 is a isopropylidene acetal protected representing the corresponding annular section. The protecting groups were necessary, as the dihydroxy functional group could interfere with numerous reactions. Fragment 2 is coupled to the side chain by Nozaki-Hiyama-Kishi reaction. The side chain could be added as vinyl iodide or enol triflate and need to be tested further for the applicability using the Nozaki-Hiyama-Kishi reaction. Esterification was done with the generated alcohol and the carboxylic acid functional group of Fragment 1. Later olefin metathesis reaction with the two terminal double bonds of the ester has to be done using Grubbs catalyst to obtain the macrolide followed by the deprotection of the isopropylidine acetal of the macrolide ring and the paramethoxybenzyl group of the side chain. Finally, oxidation of the primary alcohol of the side chain has to be done to obtain the desired carolacton in a stereoselective fashion.

The synthesis of the side chain of carolacton is discussed in this thesis in detail, starting with the construction of the relative configuration anti(C-3, C-4) / anti(C-4, C-6) followed up by

synthesizing the side chain having absolute stereochemistry anti(C-3, C-4) / anti(C-4, C-6) as shown in the Figure 24. Both relative and absolute stereoselective synthesis of the side chain is discussed in detail in the following chapters.

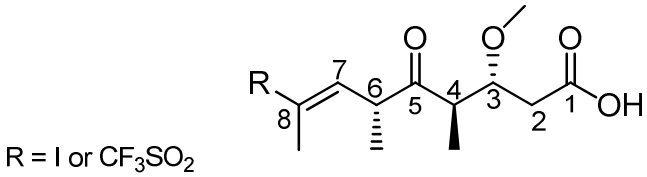


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Figure 24: Structure of the carolacton side chain

3 Syntheses

3.1 Synthesis of the side chain with defined relative configuration

In this section the synthesis of the carolacton side chain in relative stereochemistry is described. The retrosynthesis of the carolacton side chain is illustrated in Figure 25.

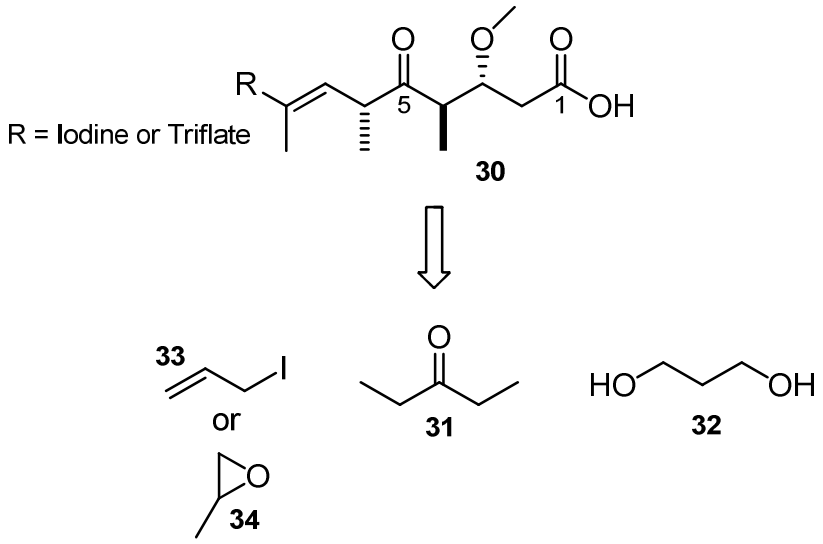


Figure 25: Retrosynthesis of the carolacton side chain in a relative configured fashion.

The target compound **30** can be constructed from 3-pentanone (**31**) as core compound. Selective reaction on the two α-carbonyl positions with two C_3 building blocks gives the target compound. One of these C_3 building blocks should be able to be transferred into an acid. This can be done by transforming 1,3-propanediol (**32**). The other C_3 building block requires a double bond on a proper leaving group at C_2. This can be achieved by starting from allyliodide (**33**) or propylene oxide (**34**).

The synthesis started from commercially available starting material 1,3-propanediol (**32**). The diol is selectively mono-protected using different protecting groups as tetrahydropyranyl, *tert*-butyldimethylsilyl, benzyl, and *para*-methoxybenzyl groups as shown in the Figure 26. The synthesis of monoprotected diols with various protecting groups was carried out in order to check the diasteromeric ratio (d.r.) of the aldol product of the corresponding protected diol. The protecting group best suited for the reaction was determined after determination of the *ds* ratio of the aldol product. The ease of removal of the protecting group was also assessed in later stages.

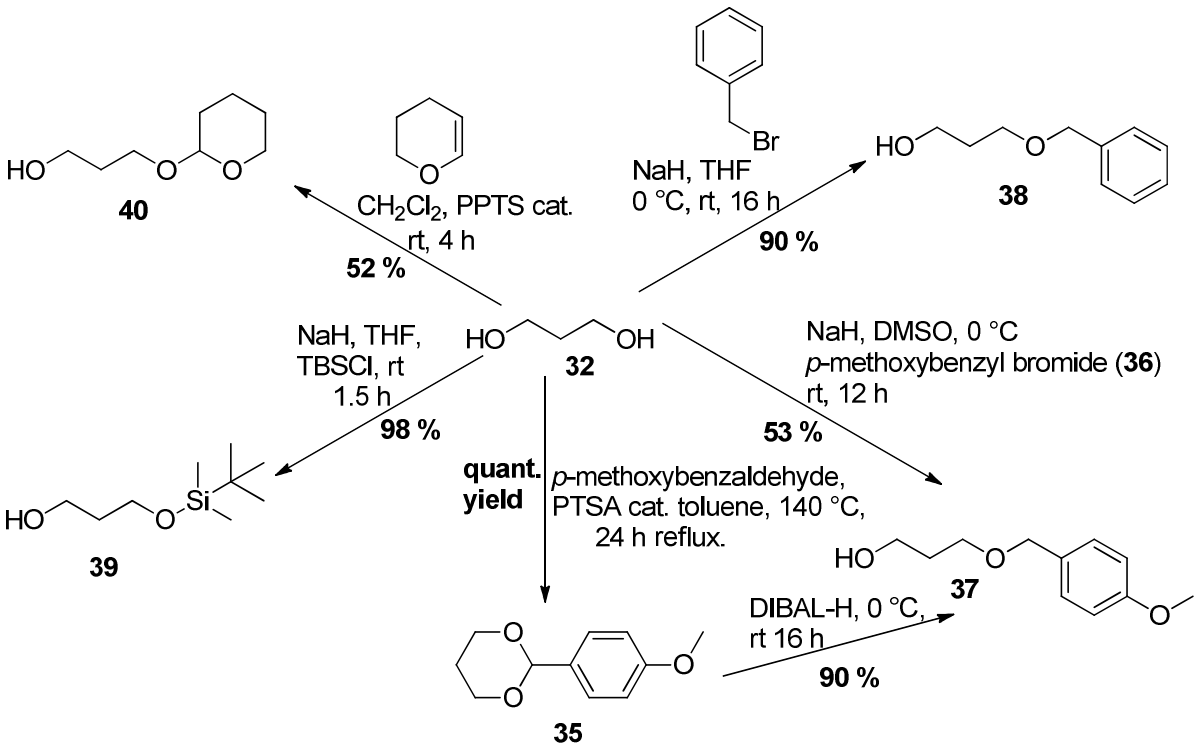


Figure 26: Mono protection of 1,3-propanediol (**32**) with different protecting groups

All the monoprotected alcohols were obtained in good (THP, PMB/NaH) to excellent yields (TBDMS, Bz). The use of NaH in the later cases avoided the formation of the diprotected product.[49–52] The fair yield of the mono PMB ether was improved according to the method of Hunter[53] and Takano[54], further modified by Uguen[55] and F.M.Cordero[56], who used a two step procedure via opening of an acetal **35** formed from 1,3-propanediol (**32**) and *para*-methoxybenzaldehyde.

The mono protected diols are oxidised to their corresponding aldehydes using swern[57], Parikh-Doering[58] as well as IBX[59] oxidation procedures as shown in the Figure 27. The oxidation of mono protected propanediol **37** with *para*-methoxybenzyl group as protecting group with IBX resulted in the best yield (80%) compared to the Parikh-Doering (70%) or swern (68%) oxidation procedures. These aldehydes are used in the Aldol reaction to generate aldol products of their corresponding protecting groups. The benzyl and *para*-methoxybenzyl protecting groups were shown to have good diastereoselective ratios compared to the other protecting group of the aldol products.

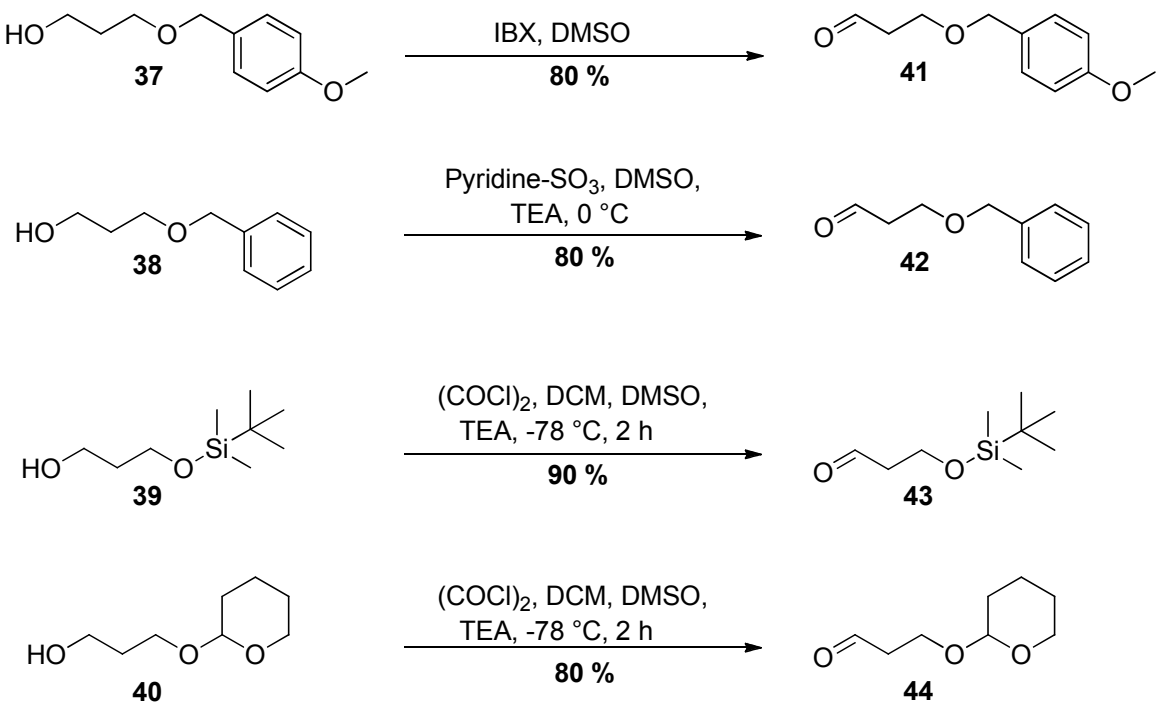


Figure 27: Oxidation of monoprotected alcohols to aldehydes

In this thesis the *para*-methoxybenzyl protecting group was preferred over the benzyl group as the cleavage of *para*-methoxybenzyl group is relatively easier than that of the benzyl group. The stability of the *para*-methoxybenzyl groups is also greater in the reactions towards the synthesis of the side chain in later stages. Benzyl groups also need hydrogenation for cleavage. This is a problem because the olefin bond might get affected in the compound. Hence, the *para*-methoxybenzyl group as protecting group is well suitable in the compound to withstand the different chemical reaction environments encountered on the way to synthesize the desired compound of interest. The next step was the aldol reaction of the protected aldehyde with pentanone (**31**) to yield the aldol product in the desired relative configuration.

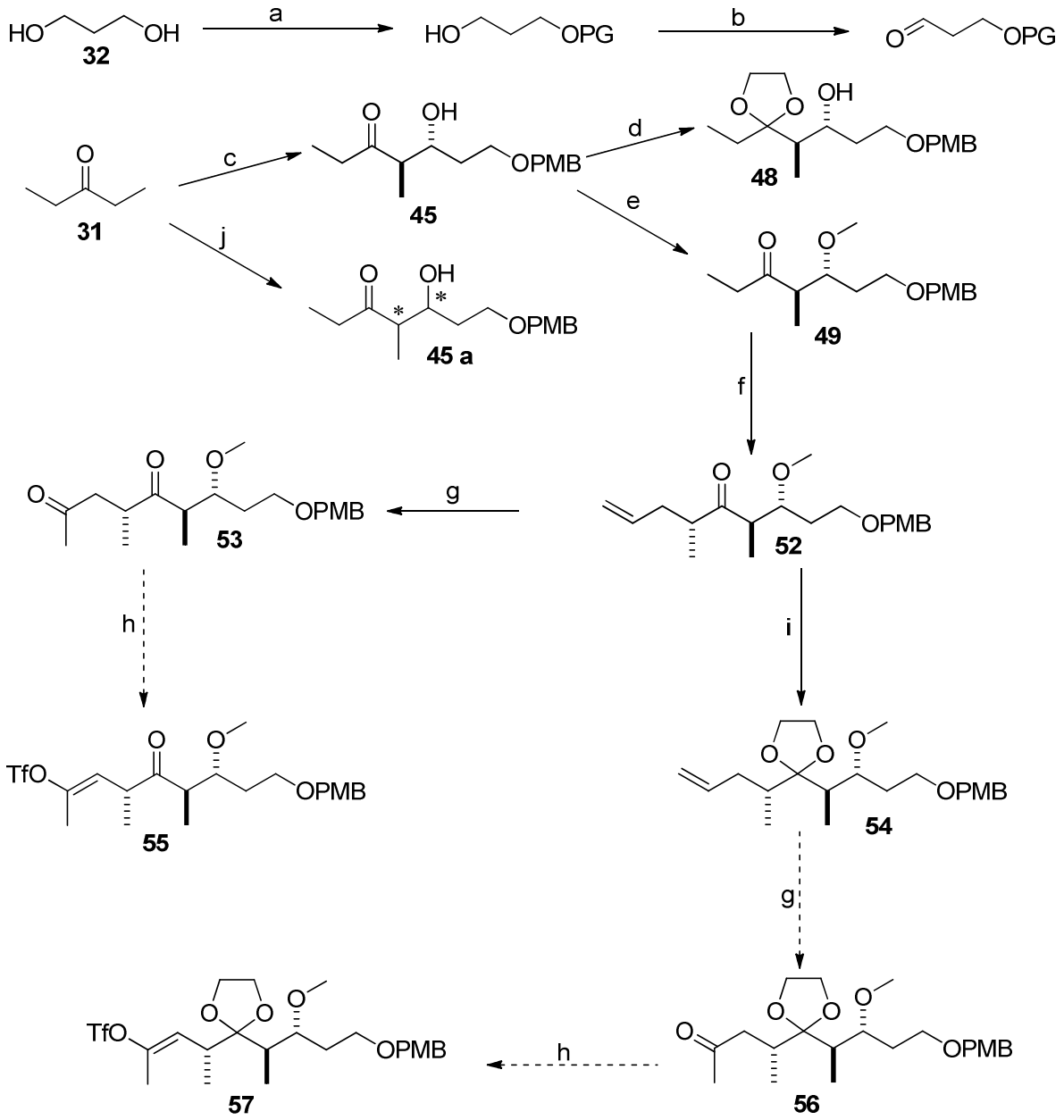
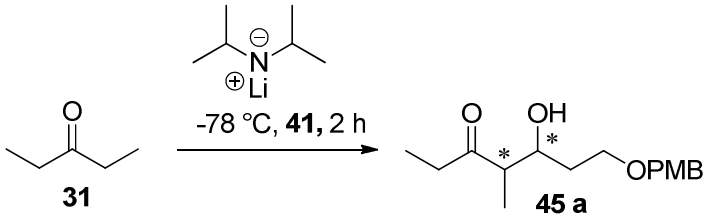


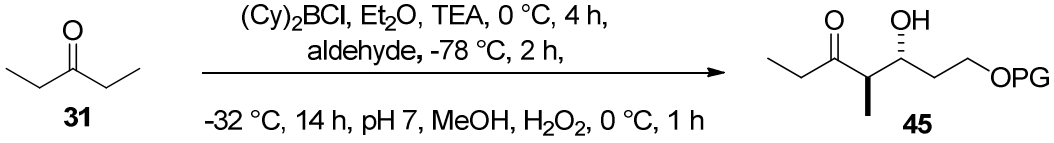
Figure 28: Reaction scheme in a relative configured fashion

Reactions and conditions: a) NaH, DMF, PMBBr, 0 °C, rt, 5 h; b) IBX, DMSO, CH_2Cl_2, 0 °C, 2 h; c) $B(Cy)_2Cl$, TEA, THF, 0 °C 4 h, -78 °C 2 h, -20 to -32 °C 12 h, pH 7 buffer solution, $MeOH/H_2O_2$, 0 °C 1 h; d) ethylene glycol, cat. PTSA, toluene, 12 h reflux; e) Proton sponge, Me_3OBF_4, CH_2Cl_2, rt, 22 h; f) NaHMDS, THF, Allyliodide, -78 °C, 4 h; g) $PdCl_2$, $Cu(OAc)_2$, O_2, atm. pressure, DMF/H_2O 8:1, 4 d; h) KHMDS, Comin's reagent, -78 °C; i) ethylene glycol, cat. PPTS, toluene, 12 h reflux; j) LDA, -78 °C, 3 h, **41**, 2 h.

The enantioselective aldol reaction using L-proline as catalyst and water as additive was not successful to synthesize the aldol product.[60] Some other catalytic enantioselective aldol reactions were reported later by Trost et al.[61] using L-proline (>30 mol%) without additive, non-proline derived catalysts and by Reiser et al. [62] using cobalt(II)-proline catalyzed direct aldol reaction. These methods were not tried.



A racemic mixture of stereoisomers **45a** was synthesized using LDA (lithium diisopropylAmide) as a base at -78 °C with aldehyde **41**. Alternatively the reaction of **31** and the different protected aldehydes **41** using Paterson aldol reaction[63] conditions afforded the anti-aldol product in a stereoselective fashion. Under these conditions dicyclohexylboron chloride ($(Cy)_2BCl$) is used as Lewis acid and triethylamine (TEA) as base. The synthesis of the aldol product with different protecting groups and their diastereoselectivity ratios are shown in the Figure 29. Although the diastereoselectivity of the aldol product containing the benzyl protecting group is higher than the aldol product containing a *para*-methoxybenzyl protecting group the latter was chosen for the next steps, because the removal of the protecting group is easier. The complete scheme of the reaction starting from the 1,3-propanediol (**32**) is shown in the Figure 28.



Aldehyde	yield	d.e.	Aldol product	Protecting group
41	89 %	96 %	**45**	*p*-methoxy benzyl
42	82 %	98 %	**46**	benzyl
43	80 %	81 %	**47**	TBDMS

Figure 29: Aldol reaction with Paterson reaction conditions. Influence of the protecting group.

Relative stereocontrol usually arise from the enolization selectivity. In this thesis the boron mediated aldol reaction is discussed. This reaction proceeds through a chair like transition state as shown in the Figure 30, where (*Z*)-boron enolates give syn-aldol products and (*E*)-boron enolates afford anti-aldol products.[64] Enolization selectivity is crucially important during the translation of the boron enolate geometry into aldol product stereochemistry. The (*E*)-enolate formation is favoured by the use of sterically demanding ligands on boron (e.g. cyclohexyl), poor leaving group (e.g. chloride) and a small amine base (e.g. triethylamine).

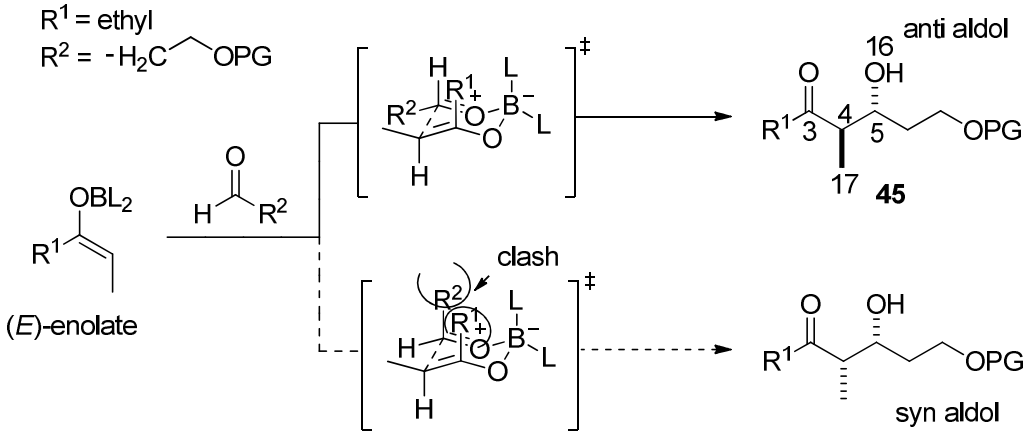
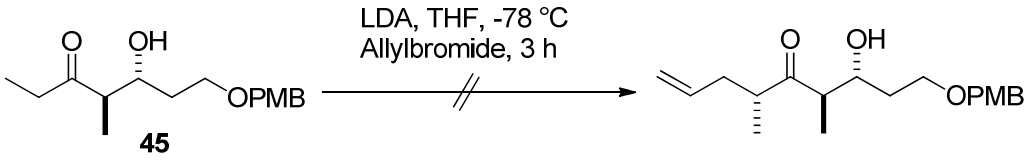


Figure 30: Chair like transition state from (*E*)-boron enolate favouring the anti-aldol product.

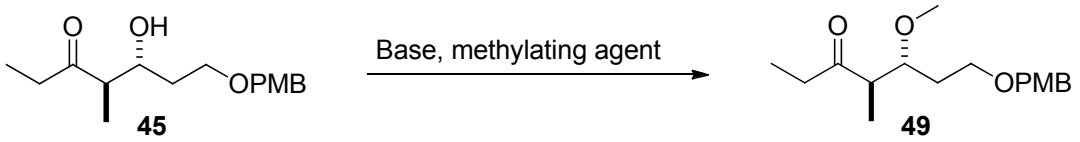
The stereochemical determination of the aldol condensation products was based on the "Stille-House" method.[65] This technique was based on the intramolecular hydrogen bond between the carbonyl oxygen and the *β*-hydroxyl proton. The hydrogen bond is thought to give rise to the predominant conformation that would lead to the observation of a corresponding predictable $^3J_{HH}$ coupling constant.[66] This rule states that the intramolecular hydrogen bond formed between the carbonyl oxygen and the *β*–hydroxyl substituent can allow the relative configuration between the two stereogenic centres to be determined by analysis of the $^3J_{HH}$ homo nuclear coupling constant between the α- and β- protons.

The $^3J_{4,5}$ homo nuclear coupling of the aldol product was found to be 9.1 Hz, measured in deuterated chloroform. Compared to the theoretical calculations within the range of 7-10 Hz predicted by the "Stille-House" method, this value is in agreement with the *threo* (anti) configured aldol product. The value found for *erythro* (syn*)* configured aldol products varies between 2 to 4 Hz.

Attempts to allylate the aldol **45** with allylbromide using LDA and LiHMDS[67] as base at -78 °C were unsuccessful. Therefore **45** was methylated first and the allylation reaction was carried out in a later stage.



Various attempts to methylate the aldol product as shown in Figure 31 resulted in the di- or tri-methylation of the aldol product rather than the methylation at the hydroxyl group. The keto group and the methylene group adjacent to keto group are methylated along with the hydroxyl group. The methylation of **48** can be accomplished, but the use of **45** with proton sponge was more effective. To circumvent this, the keto group of **45** was protected as acetal **48** to minimise the methylating side reactions. Nevertheless the methylation of the aldol product **45** was successful with very good yield using proton sponge® as the base and Meerwein salt[68] as the methylating agent starting from **45** without the need of protecting the keto group as acetal **48**.



Base (No. of eq.)	**Methylating Agent (No. of eq.)**	**Time**	**yield**
NaH (1.1 eq)	MeI (10 eq)	rt 28 h	-
NaH (4 eq)	MeI (10 eq)	rt 72 h	-
NaH (1.1 eq)	MeOTf (10 eq)	rt 4 h	-
2,6-di-*tert*-butyl-4-methylpyridine (2 eq)	MeOTf (2 eq)	reflux 14 h	-
Diisopropylethylamine (1.1 eq)	Me_3OBF_4 (1.1 eq)	rt 24 h	-
Neutral alumina 1 g	Me_2SO_4 (12 eq)	rt 33 h	-
NaOH 50 % aq. (1.7 eq)	MeI (5 eq)	rt 41 h	-
-	CH_2N_2 (excess)	rt 17 h	-
Proton sponge (15 eq)	Me_3OBF_4 (15 eq)	rt 6 h	8 %
Proton sponge (8 eq)	Me_3OBF_4 (8 eq)	rt 20 h	80 %

Figure 31: Methylation of aldol product **45** with base and various methylating agents[68,69]

The two α-methyl groups of the carbonyl group in the side chain of carolacton are stereochemically oriented towards each other in an anti-arrangement. Therefore, an

epoxide-opening reaction was advised to come to the γ–hydroxylcarbonyl arrangement of the target compound. A test reaction of the ketone **50** with epoxide **34** was carried out to find out the regio- and stereoselective outcome of the reaction. In this process the addition reaction of the metal enolate derived from the ketone **50** to the epoxide **34** gives γ–hydroxyketones. The regioselective attack of the lithium enolate derived from ketone **50** to the unsymmetrical epoxide preferably on the less substituted oxirane carbon using LiHMDS as a base in the presence of the $LiClO_4$ catalyst[70] afforded γ–hydroxy ketone **51** in 40% yield. The regio and stereoselectivity of the reaction was investigated as shown in the Figure 32. It was observed there was no predominant formation of one stereo isomer over the other indicating a racemic mixture of the product **51** and no stereoselectivity in the reaction.

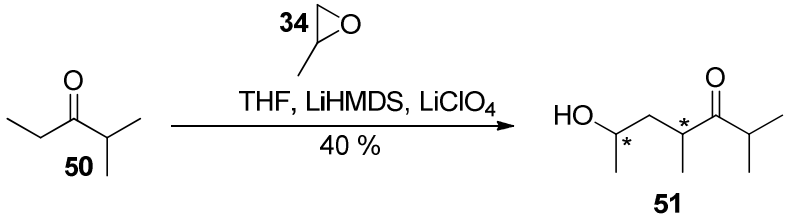


Figure 32: Reaction of **50** with 2-methyloxirane (**34**)

Based on the above result, another approach towards the side chain by proceeding with the alkylation of **49** with allyl Iodide **33** using NaHMDS as base was performed at -78 °C for 4 h to afford the allyl product **52** preferably as anti diastereomer in 40% yield with 91% (*d.e.*) (Figure 33). The sodium enolate of **49** showed good diastereoselectivity (major *anti* diastereomer) for alkylation presumably through a non-chelated intermediate.[71] The anti-configured stereochemistry was confirmed by the NMR data analysis of NOESY spectra (Figure 34); clear indication of the close spatial arrangement of the methine proton at C-4 (δ 2.69 ppm, *J* = 6.8 Hz) and methyl group at C-19 (δ 1.05 ppm, *J* = 6.8 Hz) as well as the methine proton at C-6 (δ 2.90 ppm, *J* = 7.1 Hz) and methyl group at C-20 (δ 0.95 ppm, *J* = 6.8 Hz).

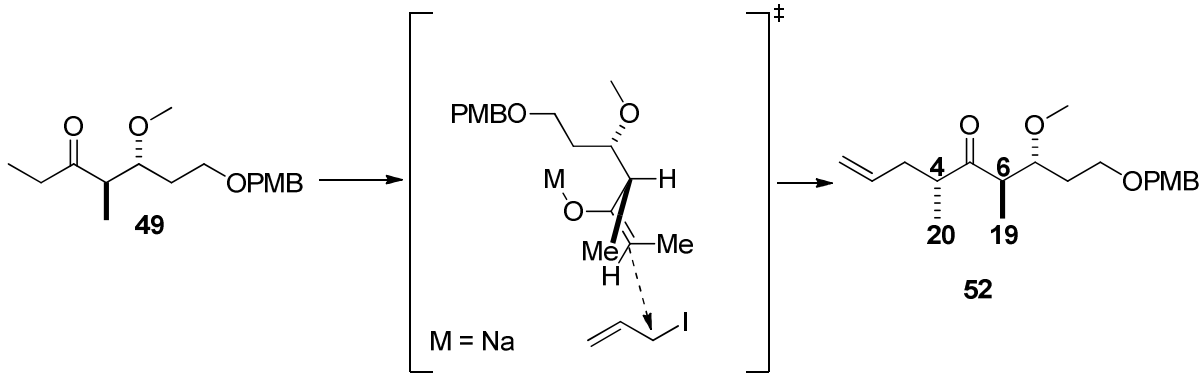


Figure 33: Reaction of **49** with allyliodide (**33**)

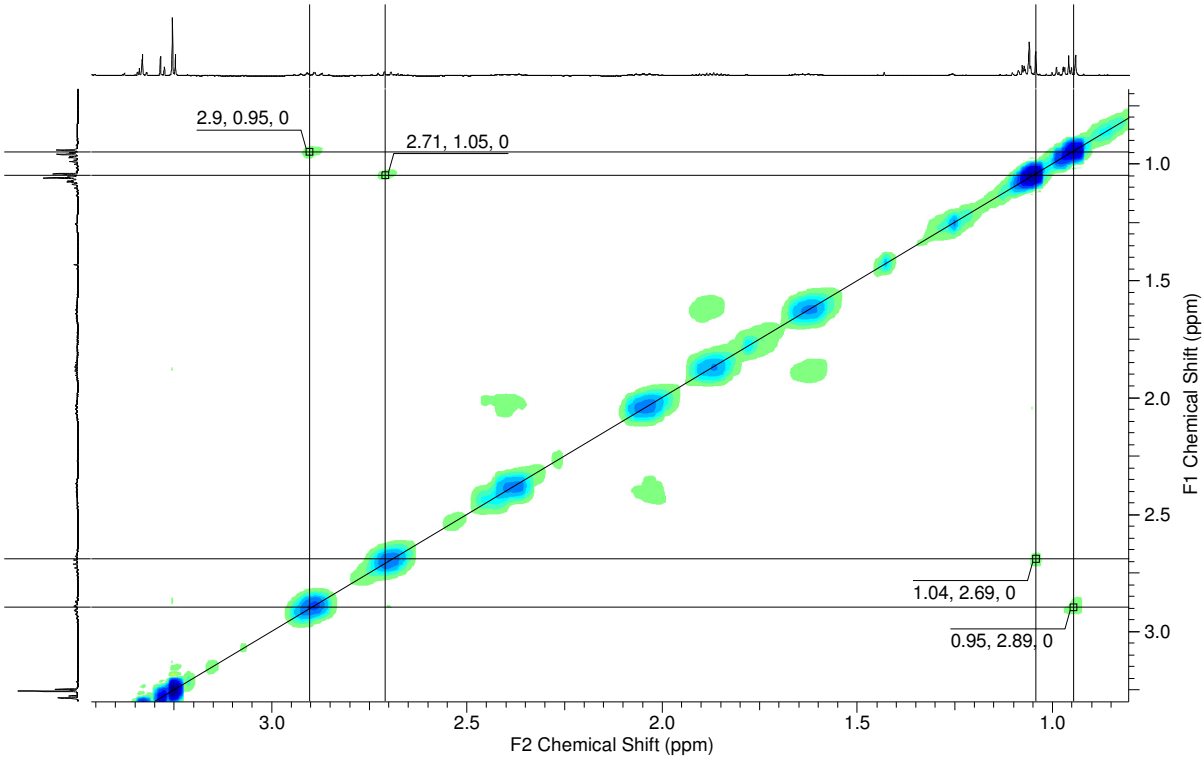


Figure 34: NOESY Spectra of **52**

The terminal alkene **52** established was the precursor of the methylketone **53**. This functional group conversion can be performed by Wacker-Tsuji oxidation.[72] However the slight modification by SMITH et al.[73] to the Wacker oxidation by using copper acetate instead of copper chloride was considered in this reaction in order to replace the strongly acidic HCl formation with weakly acetic acid byproduct during the course of the reaction (Figure 35). The terminal alkene **52** upon Wacker oxidation was transformed with O_2 at atmospheric pressure at room temperature for four days and resulted in the diketone **53** with 50% yield.

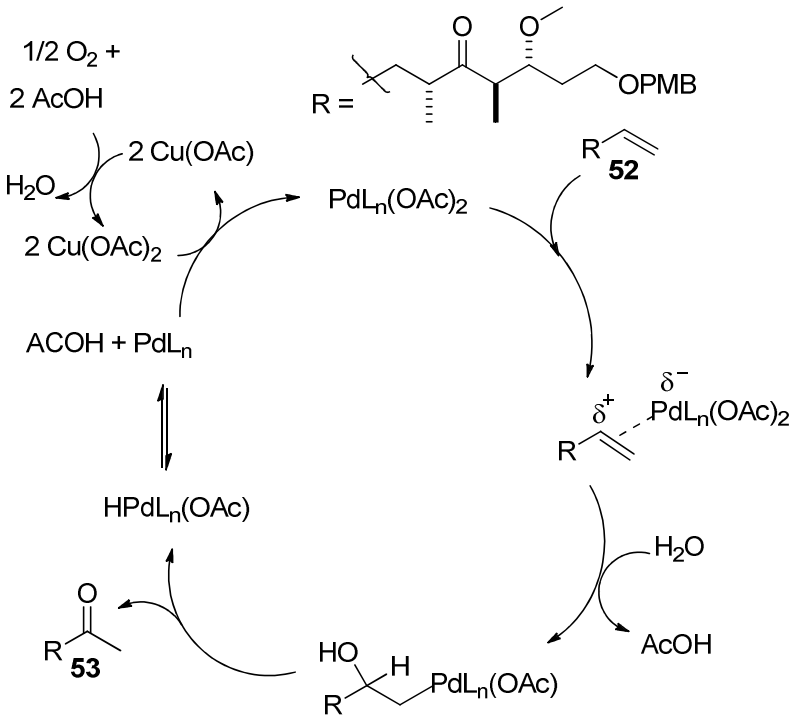


Figure 35: Schematic diagram of the palladium catalyzed Wacker-Tsuji oxidation[73]

The final transformation of the diketone **53** is formation of the triflate **55.** Nevertheless, in triflate **55** the keto group is unprotected and might get prone to side reactions in further steps of the synthesis. To avoid this, the keto group of the allyl product **52** was protected without problem to the acetal **54.** Wacker oxidation product **56** from **55** can be carried then to further steps selectively to the triflate **57** using KHMDS and comin`s reagent[74] without affecting the keto group adjacent to the methoxy group as shown in Figure 28.

3.2 Synthesis of Chiral Ketones

The chiral ketones were synthesized to synthesize the side chain stereoselectively using either Enders SAMP/RAMP method or Evans protocol explained in the following sections. The use of the SAMP/RAMP method according to the Enders protocol is explained below.

3.2.1 Synthesis of chiral ketone using Enders method

Chiral ketone **60** was synthesized from the commercially available diethyl ketone **31**, a core starting material. The great synthetic potential of metalated dimethylhydrazones as highly reactive intermediates in regio- and diastereoselective C-C bond formation reactions was well demonstrated by E. J. Corey and D. Enders in 1976.[75] They reported the asymmetric α–alkylation of ketones via the corresponding (*S*)-1-amino-2-methoxymethylpyrrolidine (SAMP) hydrazone **58a** and (*R*)-1-amino-2-methoxymethylpyrrolidine (RAMP) **58b** derivatives. The synthesis of chiral ketone **61** using Enders methodology is shown in the Figure 36.

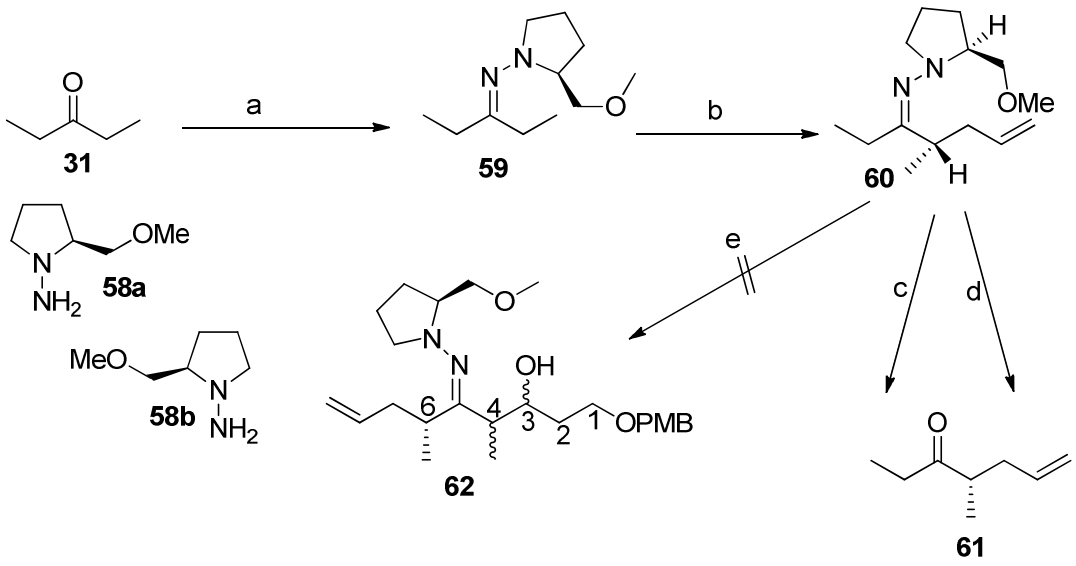


Figure 36: Reaction scheme for synthesis of the chiral ketones **61** and **62** using the SAMP pathway

Reactions and conditions: a) (*S*)-1-amino-2-(methoxymethyl)-pyrrolidine, 60 °C, 21 h; b) LDA, Et_2O, 0 °C, 4 h, allyl Iodide **33**, -110 °C, 3 h; c) MeI, 65 °C, 2 d, 4 N HCl, 5 min., pentane, 30 min.; d) $CuCl_2$, THF, 1 d; e) LDA, Et_2O, 0 °C, 4 h, **41**, -78 °C, 2 h.

The SAMP chiral auxiliary can be obtained from (*S*)-proline[76] in four steps in 58% over all yields and its enantiomer RAMP can be obtained from (*R*)-glutamic acid[77] in six steps in 35% yield. According to the Enders procedure the SAMP hydrazone was synthesized quantitatively by heating the ketone with SAMP at 60 °C for 21 h, followed by deprotonation with lithium diisopropylamide in THF. This leads to the formation of the Lithiated azaenolate that was trapped with the electophile allyl Iodide **33** at -110 °C. The reaction proceeds, from the sterically more accessible face with high diastereoselectivity as shown below to afford the allylated SAMP hydrazone (*Z*,*S*,*S*)-isomer **60** predominantly as a major isomer among the possible four stereoisomers, as shown in the Figure 37 with minor (*E*,*S*,*S*)-isomer, the geometric isomer of **60**.[78]

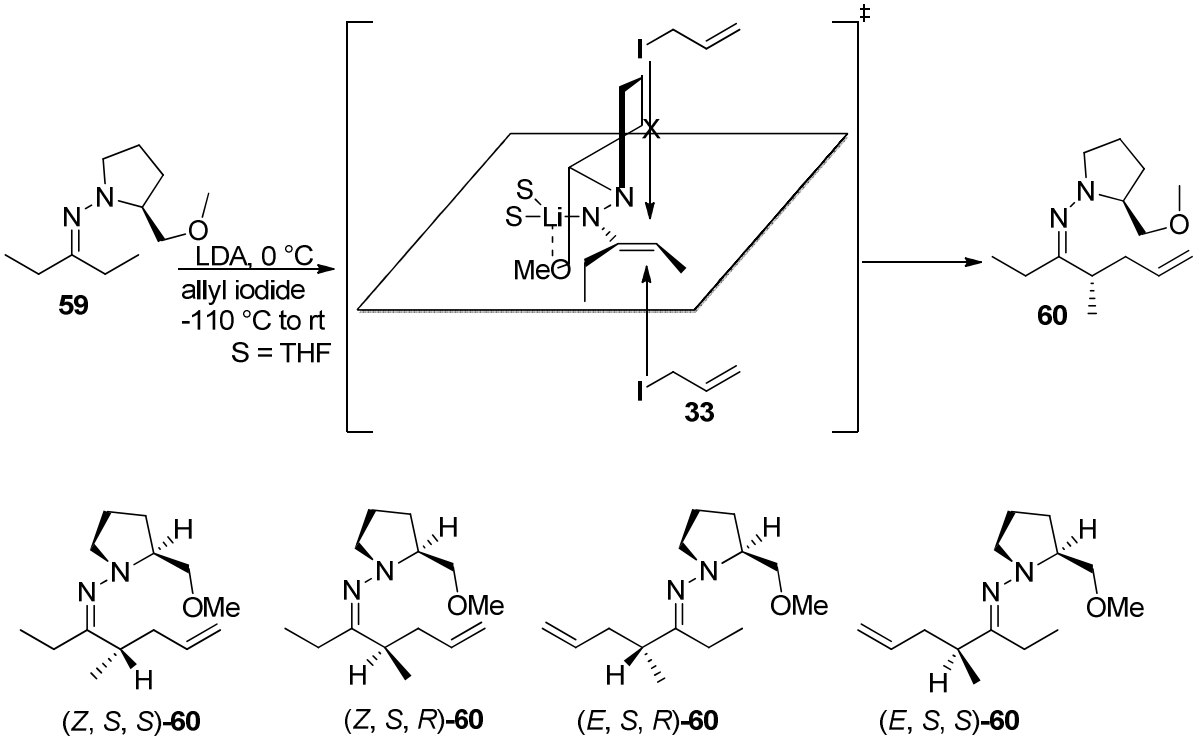


Figure 37: Possible four stereoisomers of allylated SAMP hydrazone **60**[78] and transition state

The major isomer (*Z*,*S*,*S*)-**60** was obtained in 95% yield with 98% (*d.e.*) excess. Cleavage of the SAMP hydrazone can be done through various procedures. However, limitations apply in this case due to the presence of the terminal alkene. Two procedures, the salt method and cleavage with Copper mediated hydrolysis,[79] were carried out to check out the outcome of the enantiomeric excess of the desired chiral ketone **61**. In the salt method the allylated SAMP hydrazone was treated with the excess of MeI at 60 °C leading quantitatively to the mixture of the methiodides **60a** and **b** as shown in Figure 38, which were hydrolyzed without

further purification in a two phase system (3-4 N HCl/ n-pentane) to afford the (*S*)-chiral ketone **61**. Surprisingly, the salt method furnished **61** with 60% yield and 90% *ee* compared to the 90% yield with 60% *ee* obtained with the copper mediated hydrolysis. In addition, to the above methods used for cleavage of the SAMP auxiliary, the enantiomeric excess (*ee*) could also be high by using saturated aqueous oxalic acid solution as the racemization free cleavage of the ketones. This method was widely reported to give excellent yields and high enantiomeric purity.[80] Moreover, the recovery of SAMP auxiliary would be possible with this procedure.

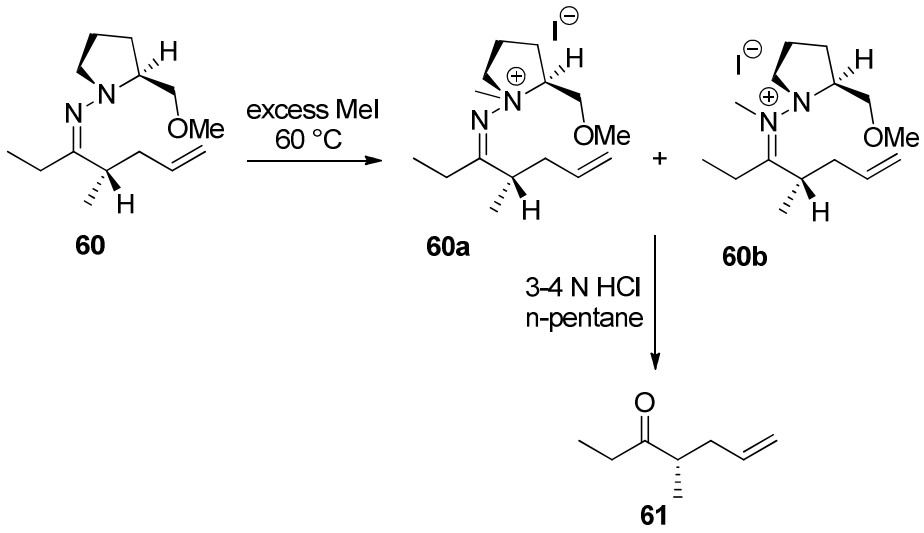


Figure 38: Salt method in a two phase reaction to obtain a chiral ketone **61**

Efforts to synthesize the carolacton side chain using the intermediate **61** without cleavage of the SAMP auxiliary by aldol reaction in the presence of LDA were fruitless when the reaction was carried out at 0 °C with LDA followed by the addition of aldehyde at -78 °C to generate the enolate; however possible reaction with LDA at -78 °C followed by the addition of the aldehyde at the same temperature could possibly lead to the product β–hydroxyhydrazone **62**, as a similar reaction was reported by D. Enders et al.[81] Switching in between the SAMP and RAMP auxiliaries could possibly lead to the desired β-hydroxyhydrazone product **62** bearing (3,4)-*anti* and (4,6)-*syn* relative configurations.[82]

3.2.2 Synthesis of chrial ketone using Evans auxiliary

3.2.2.1 Synthesis of chiral ketone 61 with terminal alkene

Because the SAMP/RAMP method proved unsuccessful, the Evans chiral auxiliary protocol was tried. The synthesis of chiral ketone **61**, using Evans methodology was started with propionyl chloride (**63**) and Evans auxiliary **64**,[83] the commercially available starting materials. The synthesis of the chiral allylketone **61** is as shown in the Figure 39.

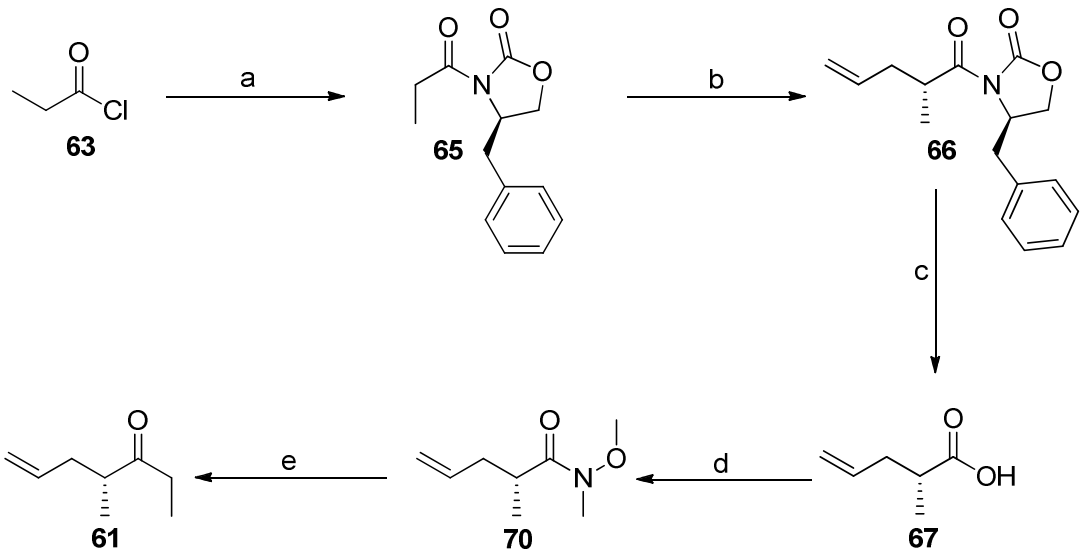


Figure 39: Reaction scheme of chiral ketone using Evans auxiliary

Reactions and conditions: a) (*R*)-4-benzyl-2-oxazolidinone **64**, THF, -78 °C, *n*-BuLi, 1 h, 0 °C, 3h; b) THF, NaHMDS, -78 °C, 1 h, allyl bromide, -45 °C, 4 h; c) THF/H_2O 3:1, H_2O_2, LiOH.H_2O, 0 °C, 2 h, $Na_2S_2O_3$, 0 °C, 20 min.; d) CH_2Cl_2, 1,1'-carbonyldiimidazole **68**, 30 min., rt, *N,O*-dimethylhyxdroxylamine hydrochloride **69**, 20 h; e) EtMgBr **71**, THF, -20 °C.

The commercially available chiral auxiliary (*R*)-benzyl-2-oxazolidinone **64** was deprotonated and subjected to propionyl chloride **63** providing the *N*-propionyl oxazolidinone **65** in excellent yield[84] with 99% yield and 99% *ee*. Asymmetric alkylation[85] of the formed product **65** was carried out in the presence of NaHMDS to form an sodium enolate and trapped with the electrophile allyl bromide which attacks from the less hindered bottom side of the enolate. The benzyl group shields the upward approach (Figure 40), to obtain the allylated product **66** in 94% yield with 99% (*d.e.*).

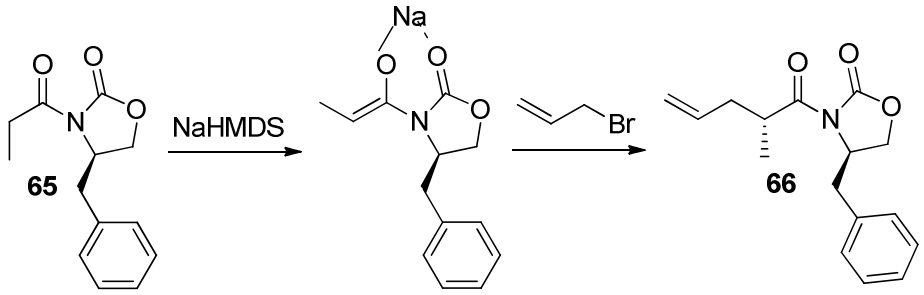


Figure 40: Reaction of the metal enolate with electrophile

The cleavage of the Evans Auxiliary with $LiOH/H_2O_2$ hydrolysis[84,86] leads to the allylated enantiomeric acid **67** in 99% yield with 98% *ee*. Recovery of the Evans auxiliary without the loss of the enantioselectivity was possible by hydrolysis. The acid was treated with 1,1'-carbonyldiimidazole (CDI) **68** at room temperature followed by the addition of *N,O*-dimethylhyxdroxylamine hydrochloride **69** using Heathcock's protocol[87] i.e; imidazolide precursor obtained from the carboxylic acid and Staab reagent (CDI) resulted in the Weinreb amide **70** in excellent yield upto 99% with 99% *ee* retaining the enantioselectivity. The Weinreb amide **70** upon Grignard reaction with ethyl magnesium bromide **71** below 0 °C afforded the desired allylated chiral ketone **61** in good yield 90% with 99% *ee*.[88,89]

3.2.2.2 Synthesis of chiral ketone 80 with internal alkyne

The synthesis of the chiral ketone **80** using Evans auxiliary with an internal alkyne was commenced by using pent-3-yn-1-ol (**72**) as the commercially available starting material. The alkyne chemistry using Evans auxiliary beside the alkene chemistry is carried out to study the introduction of the iodine group in the side chain instead of the triflate group. The schematic illustration of the synthesis of chiral ketone with alkyne chemistry consisting of internal alkyne was shown in Figure 41.

Jones oxidation[90] of the pent-3yn-1-ol (**72**) at room temperature afforded the acid **73** in moderate yield of 60% with the inverse addition method.[91,92] Introduction of the Evans auxiliary **64** to the acid group *via* one pot reaction by *insitu* generation of the anhydride with PivCl **74** instead of the synthesis of the halide of the corresponding acid **73**; and reaction of the anhydride with the lithiated species of the Evans auxiliary (*R*)-4-benzyl-2-oxazolidinone **64** afforded the Evans auxiliary product **75** in only 36% yield whereas the major by product being the Evans auxiliary protected PivCl **76**.[93]

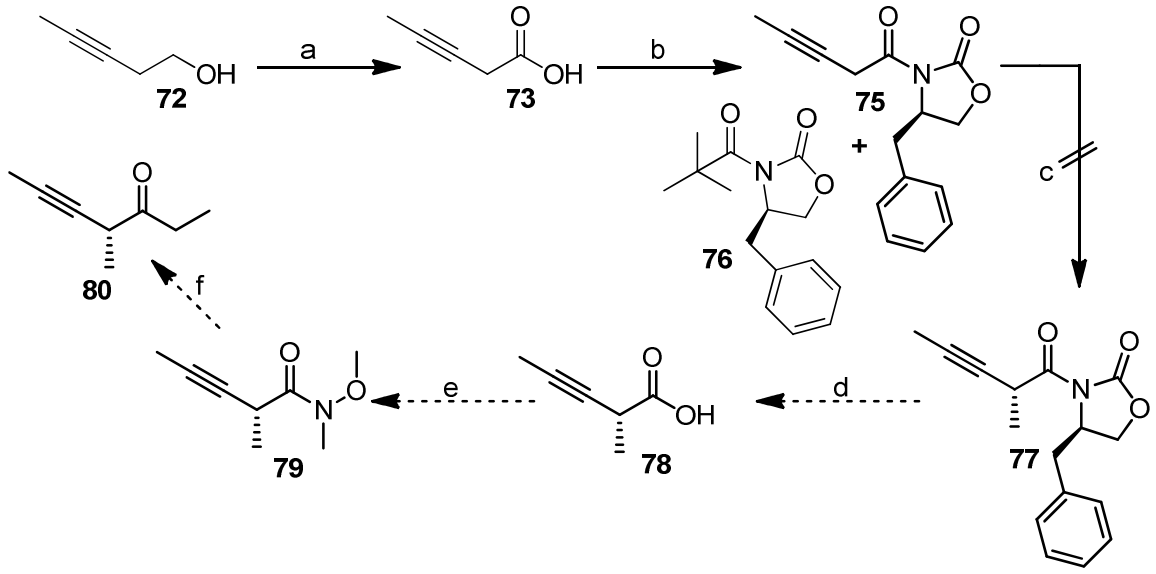


Figure 41: Reaction scheme of chiral ketone **80** with internal alkyne using Evans Auxiliary

Reactions and conditions: a) CrO_3, icebath, H_2SO_4, acetone, rt, 15 h; b) THF, Et_3N, PivCl **74**, -78 °C, 20 min., -78 °C to rt, 45 min., (*R*)-4-benzyl-2-oxazolidinone **64**, THF, -78 °C, *n*-BuLi, 15 min.; -78 °C to 0 °C, 3 h, 0 °C, 45 min., c) THF, NaHMDS, -78 °C, 1 h, MeI, -78 °C, 5 h; d) THF/H_2O 3:1, H_2O_2, $LiOH.H_2O$, 0 °C, 2 h, $Na_2S_2O_3$, 0 °C, 20 min.; e) CH_2Cl_2, 1,1'-carbonyldiimidazole **68**, 30 min., rt, *N,O*-dimethylhyxdroxylamine hydrochloride **69**, 20 h; f) EtMgBr **71**, THF, -20 °C.

The Evans auxiliary product **75** was then first treated with the NaHMDS to generate the enolate. The alkylation with methyl iodide resulted in the isomerized product **82** with terminal alkyne instead of the internal alkyne **77**. The reaction was not reliable, because often only starting materal was isolated. This led me to think over the change of the synthetic scheme to shift to the terminal alkyne instead of internal alkyne. It is explained below.

3.2.2.3 Synthesis of chiral ketone 88 with terminal alkyne

The synthesis of the chiral ketone with terminal alkyne as shown in Figure 42 was started with the commercially available starting material pent-4-ynoic acid **81**. Introduction of the Evans auxiliary *via* one pot synthesis by *insitu* generation of the anhydride of the corresponding acid was done instead of traditional synthesis of the halide of the corresponding acid.[94] The anhydride was immediately at -78 °C treated with the lithiated species of the (*R*)-4-benzyl-2-oxazolidinone **64** to afford the Evans auxiliary product **82** in excellent 94% yield with 99% *ee*. Only traces of the byproduct **76** were formed.

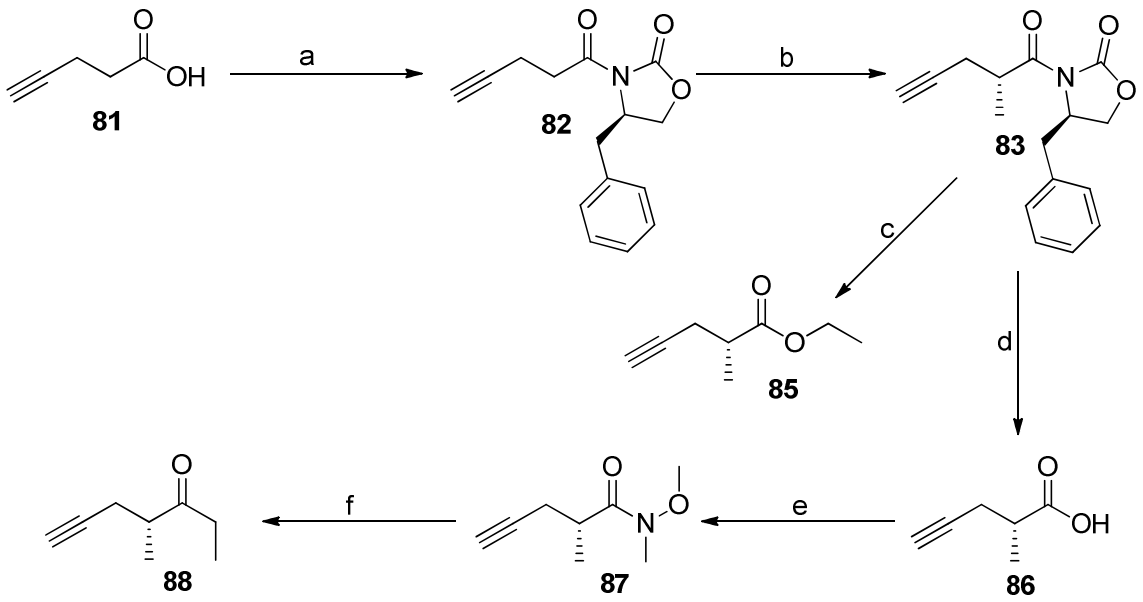


Figure 42: Reaction scheme of chiral ketone **69** with terminal alkyne using Evans auxiliary

Reactions and conditions: a) THF, Et_3N, PivCl **74**, -78 °C, 15 min., (*R*)-4-benzyl-2-oxazolidinone **64**, THF, -78 °C, *n*-BuLi, 30 min.; b) THF, NaHMDS, -78 °C, 1 h, MeI, -78 °C, 4.5 h; c) EtOH, $Ti(OEt)_4$ **84**, 20 h reflux; d) THF/H_2O 3:1, H_2O_2, LiOH.H_2O, 0 °C, 2 h, $Na_2S_2O_3$, 0 °C, 20 min.; e) CH_2Cl_2, 1,1'-carbonyldiimidazole **68**, 30 min., rt, *N,O*-dimethylhyxdroxylamine hydrochloride **69**, 20 h; f) EtMgBr **71**, THF, -20 °C.

Generation of the enolate of the Evans auxiliary with NaHMDS and then treated with methyl iodide at -78 °C resulted in the methylated product **83** in 85% yield with 99% (*d.e.*). However, treatment of propargyl bromide **89** with propionyl-oxazolidinone **65** in the presence of LDA and HMPA[95] afforded the product **90** with the *syn* configuration in 78% yield as single diastereomer; the absolute configuration was confirmed by the X-ray crystallography (Figure 43). By inversing the Evans auxiliary in the reaction with propargyl bromide **89** in the presence of LDA and HMPA would have afforded the desired configured alkylated product **83**.

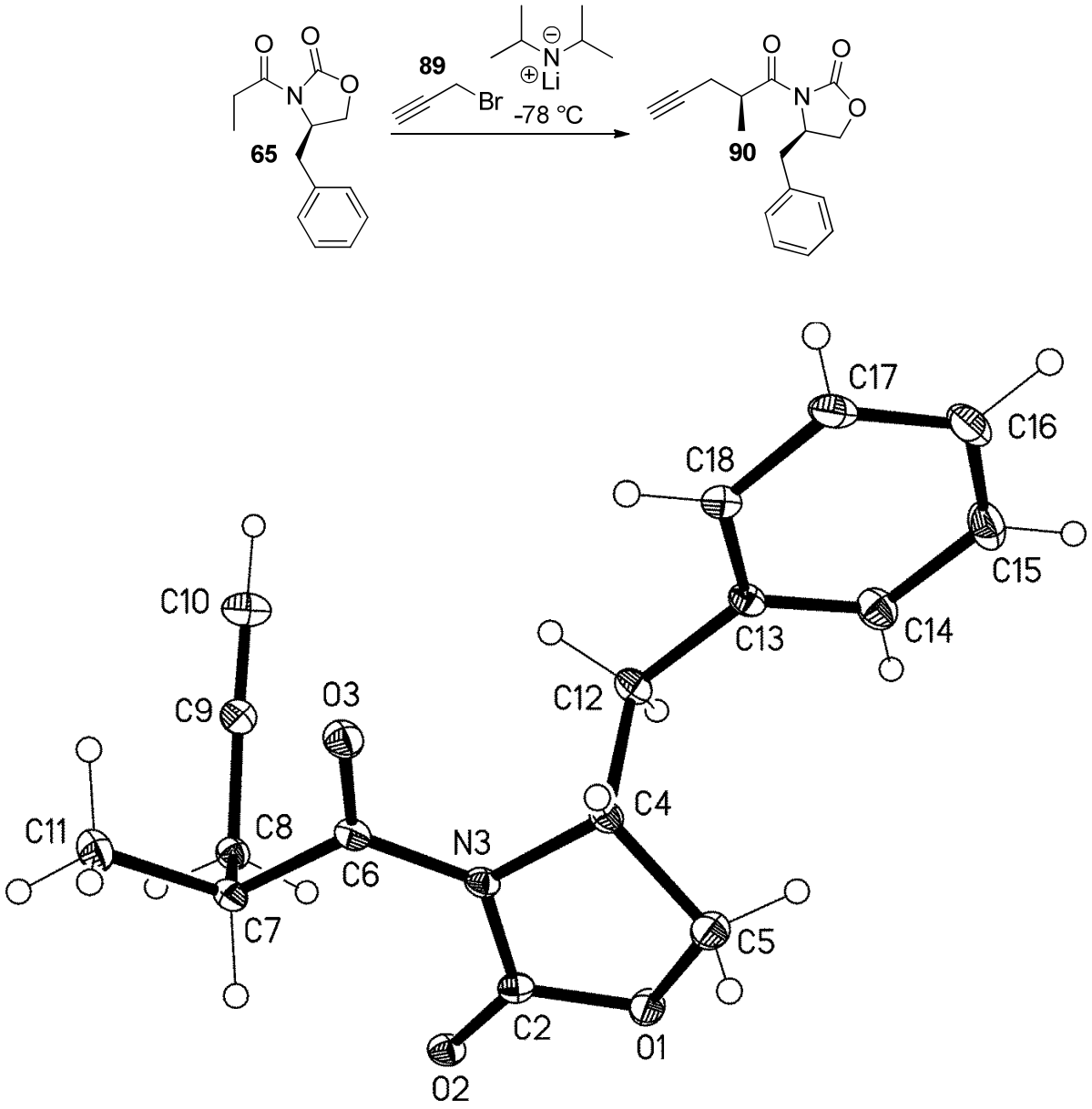


Figure 43: X-ray crystal structure of methylated methylated Evans product **90**

The generated alkylated product **83** upon $LiOH/H_2O_2$ hydrolysis resulted in the cleavage of the Evans auxiliary **64** leaving acid **86** in 85% yield with 96% *ee*. The cleavage of the auxiliary **64** to obtain the ester **67** was done by refluxing the alkylated Evans product **83** with $Ti(OEt)_4$ **84** in ethanol, sacrificing the recovery of the Evans auxiliary **64** in 85% yield with 99% *ee*. This was performed to check the *ee*. The analysis of the product by chiral GC allowed to check whether any epimerization during the hydrolysis reaction using $LiOH/H_2O_2$ took place. Fortunately, as there was no any epimerized product formed during the hydrolysis reaction, this reaction scheme was carried further *via* acid **86**. Transformation of the acid **86** into the Weinreb amide **87** using **68** and **69** afforded the amide **87** in 80% yield

with 98% *ee*. Grignard reaction of the product with ethyl magnesium bromide **71** afforded the chiral ketone **88** with terminal alkyne in 70% yield with 98% *ee*.

The chiral ketone **80** with the internal alkyne was synthesized by isomerizing the chiral acid **86** by treatment with KO*t*Bu in DMSO[96] in 54% yield and without any observed racemization of the product (Figure 44). The isomerized chiral acid **78** with internal alkyne was transformed into the Weinreb amide **79** in 78% yield and 99% *ee* in micro scale (mg quantity). Grignard reaction of the amide with ethylmagnesium bromide **71** below 0 °C afforded the desired ketone **80** with internal alkyne in 50% yield.

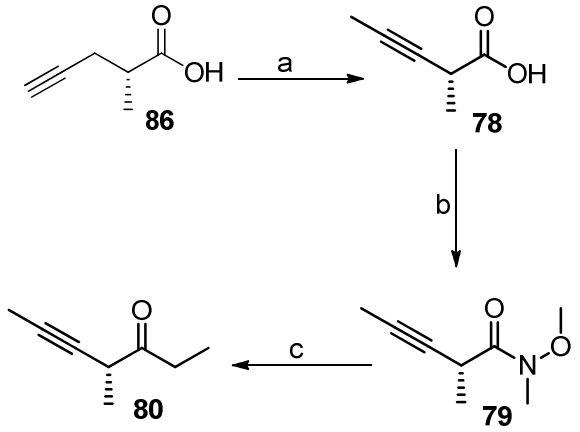


Figure 44: Reaction scheme for the chiral ketone **80** with internal alkyne by isomerization of the chiral acid **86**

Reactions and conditions: a) KO*t*Bu, DMSO, rt; b) CH_2Cl_2, 1,1'-carbonyldiimidazole **68**, 30 min., rt, *N,O*-dimethylhyxdroxylamine hydrochloride **69**, 20 h; c) EtMgBr **71**, THF, -20 °C.

3.3 Absolute configuration

The synthesis of the carolacton side chain was mentioned in chapter **3.1** with respective to the relative configuration whereas here the asymmetric synthesis of the side chain of carolacton with absolute configuration was reported. The synthesis started from utilizing the above synthesized (*S*)-chiral ketone **61** obtained from the SAMP methodology (chapter **3.2.1**) with achiral aldehyde **41** using Paterson aldol reaction[97] as shown in the Figure 45.

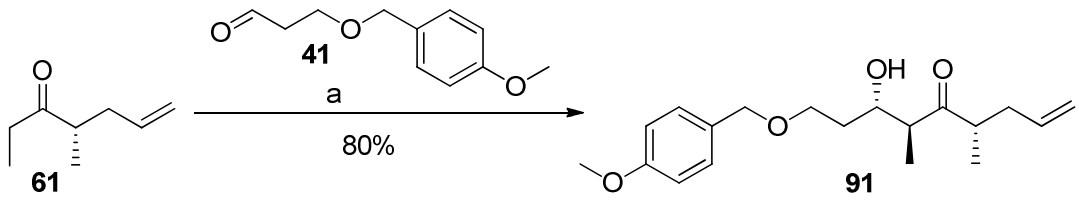


Figure 45: Aldol reaction of chiral ketone **61** with achiral aldehyde **41**

Reactions and conditions: a) $B(Cy)_2Cl$, TEA, THF, 0 °C, 4 h, -78 °C, **41**, 2 h, -20 to -32 °C, 12 h, pH 7 buffer solution, MeOH/ H_2O_2, 0 °C, 1 h.

The aldol reaction of an aldehyde with metal enolate creates two new chiral centres in the product molecule; and this lead to the four possible steroisomers as shown in the Figure 46.

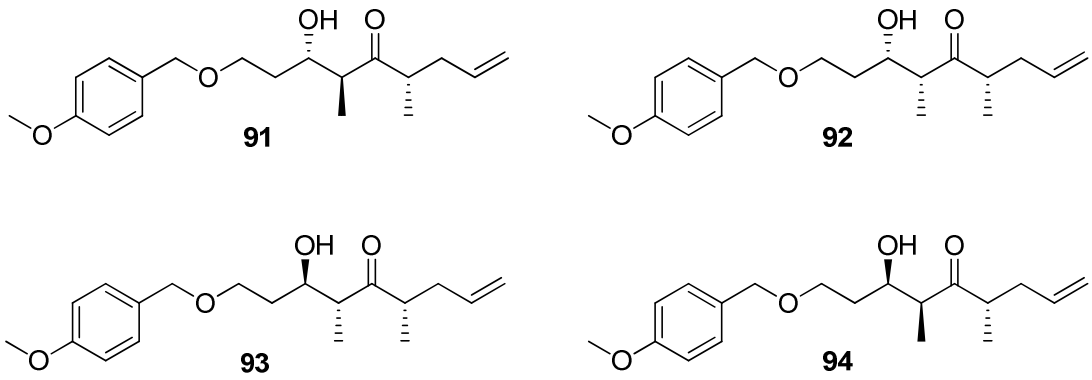


Figure 46: Stereoisomers of the aldol product of chiral ketone **61** with achiral aldehyde **41**

The *anti*-configured stereoisomers **91** and **93** are the result of the boron mediated aldol reaction resulting from the (*E*)-enolate, whereas the stereoisomers **92** and **94** obtained from the (*Z*)-enolate show *syn*-configuration (Figure 46).

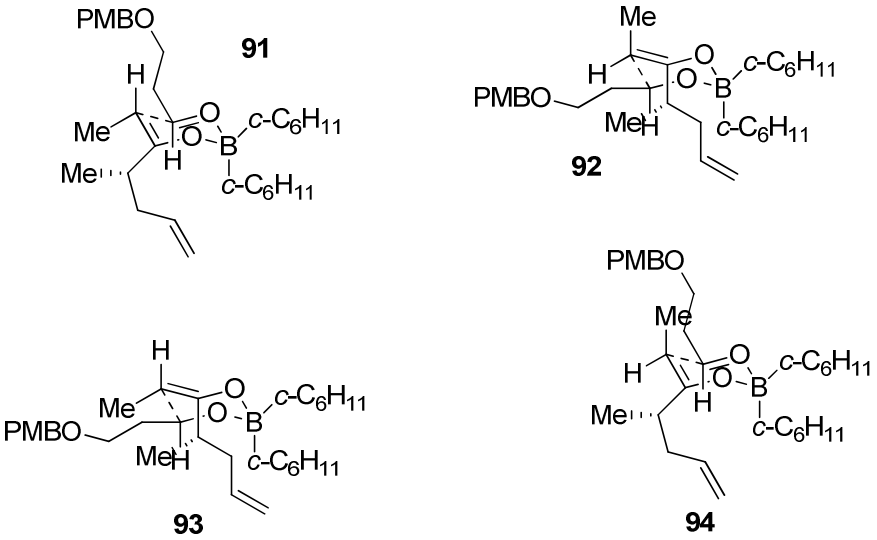


Figure 47: Chair conformations of the stereoisomers of the Aldol product

From the above Zimmerman-Traxler model as shown in the Figure 47 the (*Z*)-enolate geometry tends to give 6,7-*syn*-product **92** & **94**, whereas the (*E*)-enolate gives the 6,7-*anti*-product **91** & **93**. The rational of the high stereo-outcome is that the dicyclohexylboron enolate having relatively short metal-oxygen bonds and this is essential for maximizing the 1.3-diaxial interactions in the transition states. The *opmb*–moiety occupies a more stable transition state, a pseudo equatorial position leading to the aldol product with high stereoselectivity preferably **91** in *anti*-configured and **94** in *syn*-configured.

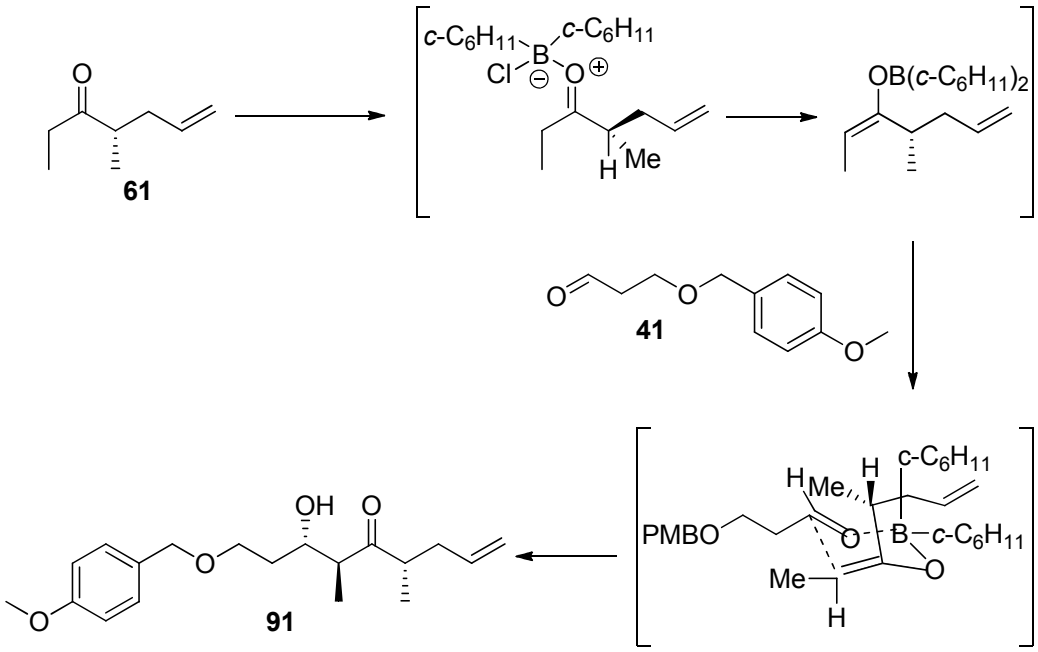


Figure 48: Aldol reaction of chiral ketone **61** with achiral aldehyde **41**

The combination of the bulky boron ligands on cyclohexyl, a boron chloride derivative and an unhindered base such as triethyl amine proved to be optimal for the stereoselective synthesis of *trans*-enolates for wide variety of ketones reported by Paterson utilizing the Brown enolization procedure.[64] As shown in the Figure 48 enolization of (*S*)-chiral ketone **61** with $(c\text{-}C_6H_{11})_2BCl/Et_3N$ affords *trans*-enolate which smoothly adds to the aldehyde **41** to give *anti*-aldol product **91** with the diastereoselectivity (d.r. = 2:1 *anti*/*syn*).

The configuration of the C-4 was retained as planned in the synthesis and the configuration of C-7 was assigned after the Mosher ester analysis. Based upon the configuration of the C-4 and C-7 the possible configuration at C-6 is revealed by the relative configuration by the NMR vicinal (^3J) coupling constants.

3.3.1 Assignment of the C-7 configuration of Aldol product via Mosher ester NMR analysis

The absolute configuration at the hydroxyl group of the aldol product containing the two inseparable diastereomers was determined by synthesizing the Mosher esters with chiral derivatizing agents (CDA) (*R*)-MTPACl **95** and (*S*)-MTPACl **96** as shown in the Figure 49.

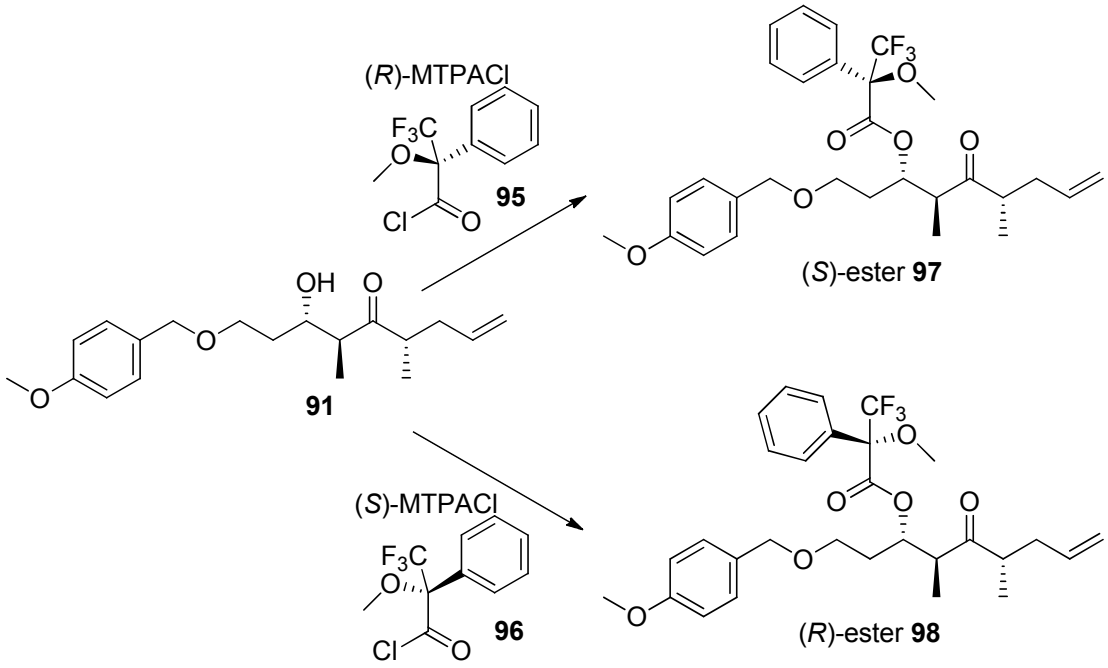
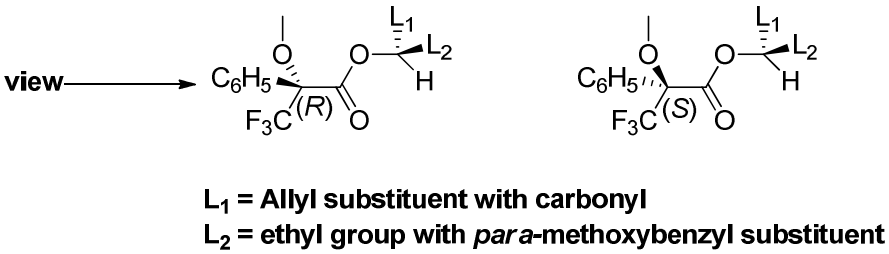


Figure 49: Reaction of aldol product **91** with CDA's to the corresponding Mosher esters

MTPACl (α-methoxy-α-trifluoromethyl-α-phenylacetic acid chloride) is most commonly used derivatizing reagent for the determination of the absolute configuration of secondary alcohols by NMR. This methodology was first reported by Mosher in 1973.[98] The procedure starts with the esterification of the aldol product with the two enantiomers of the MTPACl. The aldol product was treated with (*R*)-MTPACl and (*S*)-MTPACl in the presence of triethyl amine and catalytic amount of DMAP to transform into the (*S*)- and (*R*)-esters, respectively.[99] The (*S*)-ester **97** and the (*R*)-ester **98** were purified with flash column chromatography, followed by acquiring and comparing the NMR spectra of the two diastereomeric esters.

The two substituent's adjacent to each side of the MTPA chiral site at C-7 were designated as L_1 (the side chain with allylic group with steric bulkier methyl groups) whereas L_2 (the side chain with the protecting group *para* methoxy benzyl group) and the differences in the chemical shift were represented by Δδ; a sign of the parameter either + or –, provides the information about the configuration. Later a new methodology called as the modified Mosher Method was developed by Kakisawa and coworkers to evaluate the $\Delta\delta^{RS}$ data.[100] This

procedure involves the analysis of all of the protons in the molecule, so that a representative sign for $\Delta\delta^{RS}$ of the substituents L_1 and L_2 can then be adopted based on the majority of the protons for each substituent.[101]



L_1 = Allyl substituent with carbonyl
L_2 = ethyl group with *para*-methoxybenzyl substituent

Figure 50: Preferred conformation of product **91** with CDA's

In the above Figure 50 in the (*S*)-mosher ester the protons in the L_1 portion are more highly shielded (and appear further up field), and those in the L_2 moiety are less highly shielded (and appear further down field). The reverse is true in the case of (*R*)-mosher ester. Thus we expect $\Delta\delta^{RS}$ to be negative for L_2 and positive for L_1. By the NMR interpretation it was clear that L_1 substituent appear at upfield in the case of (*S*)-mosher ester whereas the L_2 substituent at downfield in the (*S*)-mosher ester. Calculating the $\Delta\delta^{RS}$ values and using the Cahn-Ingold-Prelog (CIP)-convention the absolute configuration of the carbinol center in the aldol product was assigned as (*S*) configured, as shown below in Table 1 and Figure 51. The chemical shift of CF_3 in ^{19}F NMR was having δ value at -71.58 ppm for (*R*)-MTPA ester and -71.55 ppm for (*S*)-MTPA ester referring the integrals the *d.e.* was calculated as 24%.

Table 1: $\Delta\delta^{RS}$ values of the MTPA ester

L_1: *Allyl group*	**δ** (ppm)	L_2: *ethyl with PMB group*	**δ** (ppm)	**MTPA Config.**
C3	1.82 1.75 +0.07	C8	1.90 1.98 -0.08	*R* *S*
C4	2.70 2.68 +0.02	C9	3.35 3.46 -0.11	*R* *S*
C10	1.06 1.03 +0.03			*R* *S*
C11	1.09 0.98 +0.11			*R* *S*

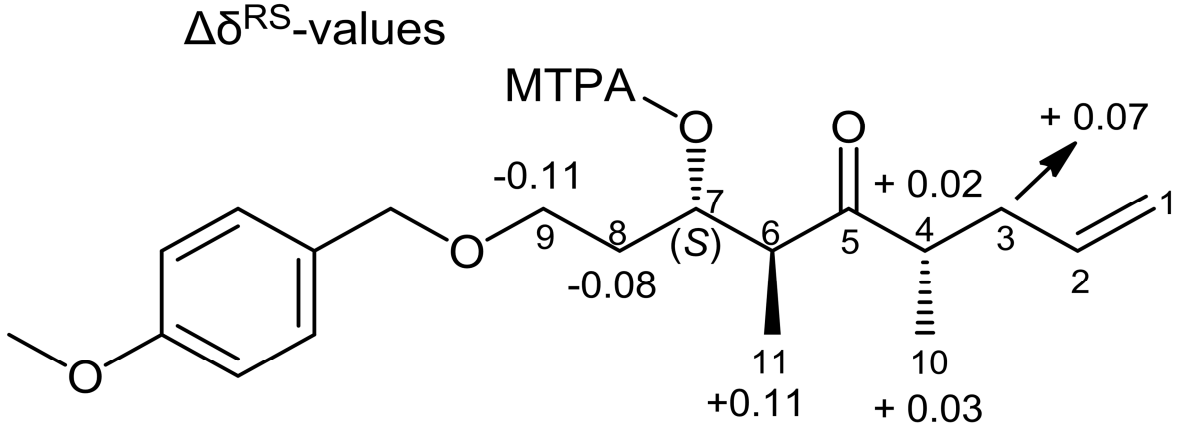


Figure 51: $\Delta\delta^{RS}$ values of the MTPA ester of aldol product **91**

The absolute configuration at C-7 was assigned as (*S*)-configured *via* modified Mosher method and the methyl group adjacent to the allyl group having (*S*) configured obtained from the (*S*)-chiral ketone **61** the assignment of the configuration at C-6 was (*S*). The diastereoselective aldol reaction produced the diastereomer with *anti*-configuration with $^{3}J_{H\text{-}6,\,H\text{-}7}$ = 7 Hz, Figure 52; shows the ^{1}H-NMR spectrum of the aldol product **91** as proven by

the earlier reaction. The proton at C-6 at δ 2.79 ppm was relatively *anti*-configured with both of the protons at C-7 at δ 3.91 ppm and C-4 at δ 2.71 ppm having the coupling constant *J* = 9.6 and 7.1 Hz respectively.

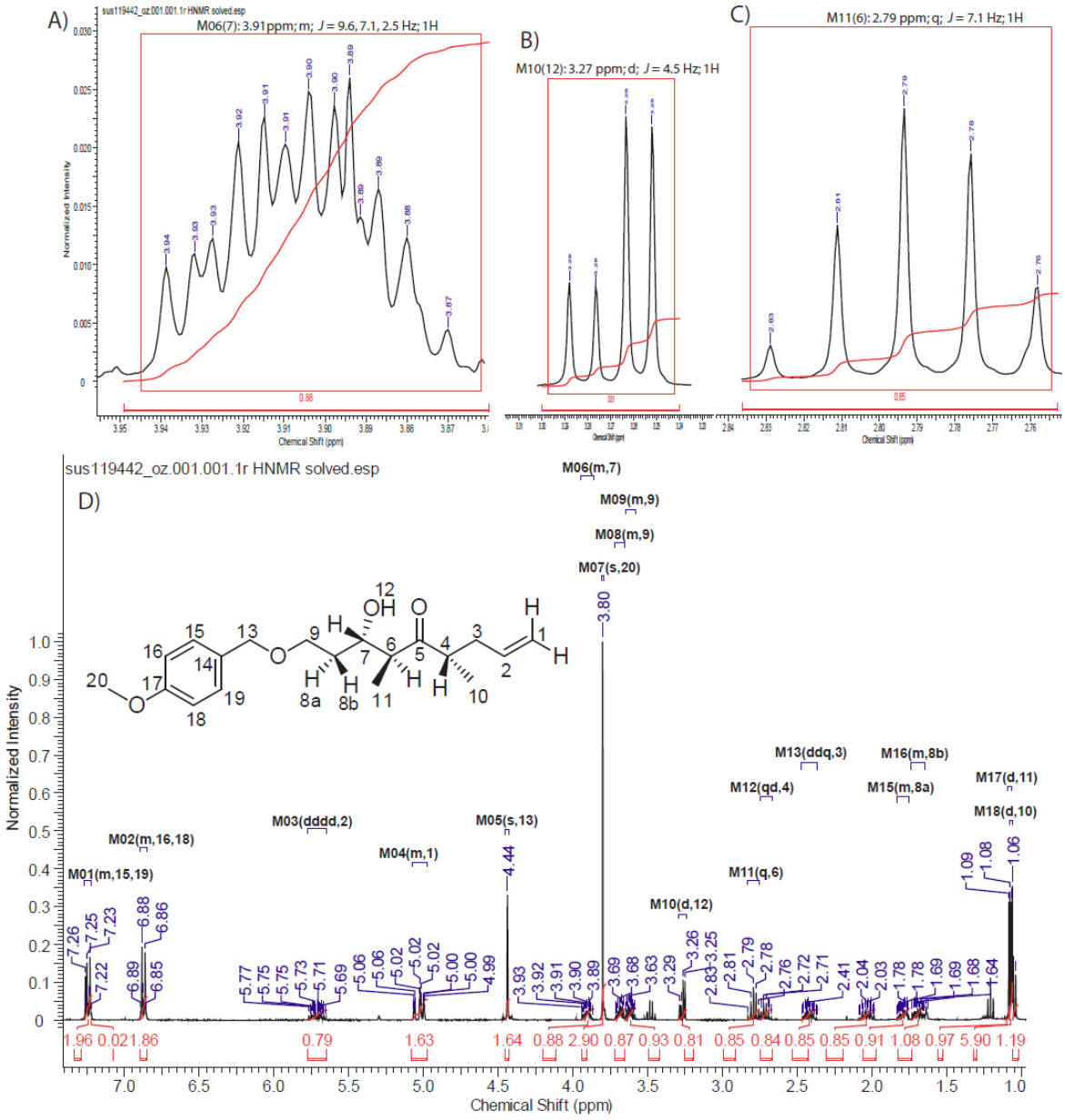


Figure 52: ^{1}H-NMR of the aldol product **91**

A) Multiplet of *H*-7 at δ 3.91 ppm; **B)** doublet of *H*-12 (-*OH* group) at δ 3.27 ppm; **C)** quartet of *H*-6 at δ 2.79 ppm **; D)** Complete ^{1}H-NMR spectrum of the aldol product **91**.

In the above spectra the multiplet (M06) of *H*-7 is having the splitting pattern of doublet of doublet of triplets (ddt) at δ 3.91 ppm with the coupling constant, *J* = 9.6, 7.1 and 2.5 Hz. The hydroxyl group (-O*H*) multiplet (M10) at δ 3.26 ppm shows the splitting pattern of

doublet (d), as well as the minor diastereomer at C-7 at δ 3.29 ppm; both isomers having the coupling constant, *J* = 4.5 Hz. The multiplet (M11) of the *H*-6 shows the splitting pattern of quartet of doublets (qd) at δ 2.79 ppm with coupling constant, *J* = 7.1 Hz.

The aldol product **91** upon methylation[102] with the meerwein salt[103] and proton sponge afforded the methoxy product **99** in 80% yield.

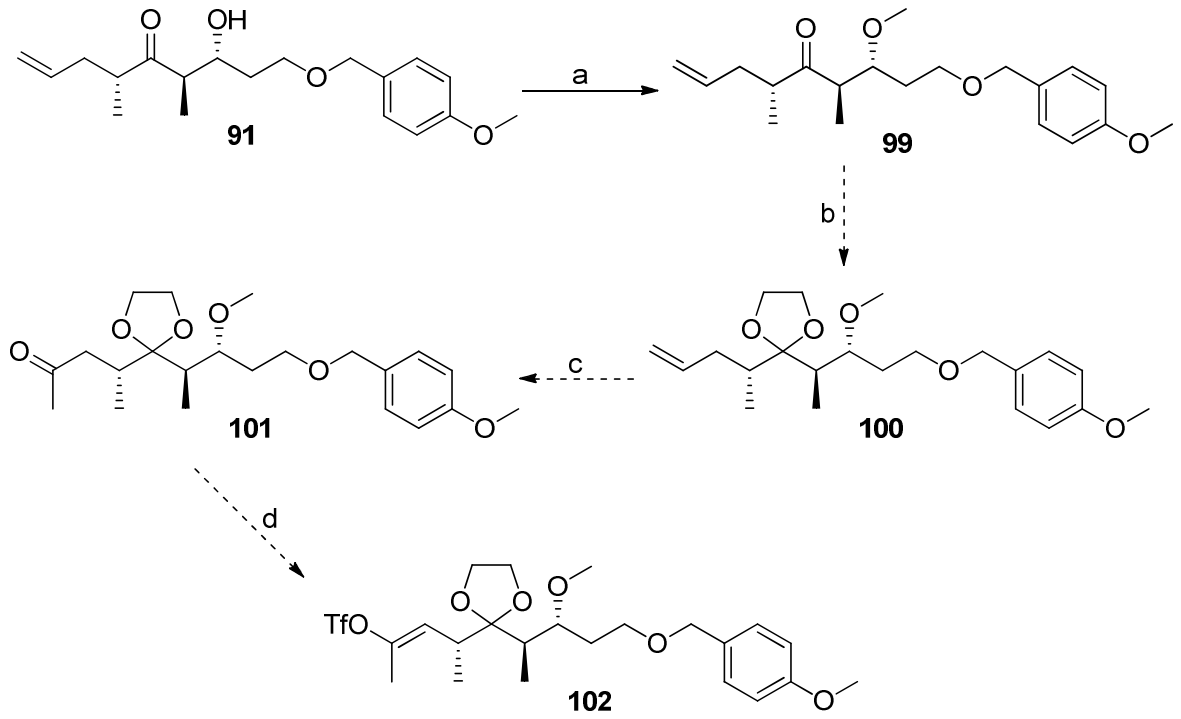


Figure 53: Reaction scheme for the side chain of carolacton *via* asymmetric synthesis

Reactions and conditions: a) Proton sponge®, Me_3OBF_4, CH_2Cl_2, rt, 22 h; b) ethylene glycol, cat. PTSA, toluene, reflux; c) $PdCl_2$, $Cu(OAc)_2$, O_2, atm. pressure, DMF/H_2O 8:1; d) KHMDS, Comin's reagent, -78 °C.

The future prospects of the reactions are to protect the keto functional group of **99** as dioxalane **100** followed by Wacker oxidation to ketone **101** and finally the generation of the triflate **102**[74] as shown in Figure 53; readily available for the Nozaki-Hiyama-Kishi reaction[104] with the aldehyde from fragment 2.

3.4 Synthesis of the carolacton side chain using epoxide pathway

Another approach towards the side chain used an epoxyalcohol-aldehyde rearrangement as key step. The aldehyde **41** served as starting material in this alternative synthesis *via* Sharpless asymmetric epoxidation as shown in the Figure 54.

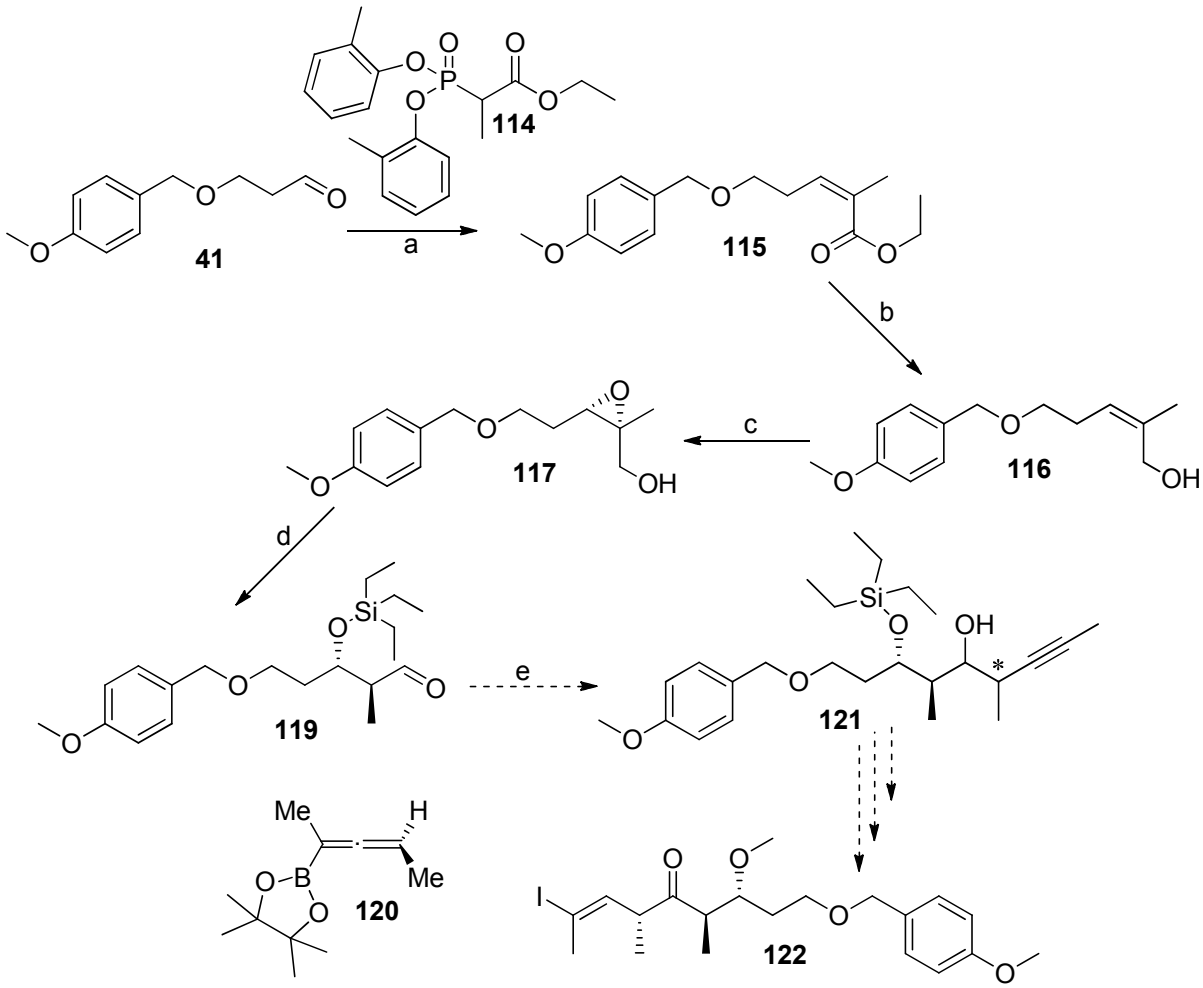


Figure 54: Reaction scheme of epoxide pathway **via** *cis*-α,β-unsaturated α-methyl ethyl ester **115**

Reactions and conditions: a) THF, **114**, 0 °C, NaI, DBU, 10 min., -78 °C, 2 h; b) Et_2O, LAH, 0 °C, 15 min.,Na_2SO_4, 0 °C, 15 min., c) DCM, 4 A° mol. sieves, Ti(O*i*Pr)$_4$, (-)-DET, -30 °C, 30 min., TBHP, -20 °C, 3 h; d) DCM, 4 A° mol. sieves, DIPEA, rt, 10 min., TESOTf **118**, -42 °C, 1.5 h; e) boronated allene **120**.

For the planned enantioselective Sharpless epoxidation a (*Z*)-configured ester was necessary. Therefore, the Still-Gennari procedure reported to be (*Z*)-selective was used. The required phosphonate was synthesized as shown in Figure 55. Dichloroethyl phosphite **103** as starting material was reacted with triflouroethanol and triethylamine in THF, thus forming bis(triflouroethyl)ethylphosphonate **105**. The second step involves the reaction of **105** by reacting it with an enolate formed from the reaction of ethyl chloroformate and a lithium amide to give bis(triflouroethyl)phosphonate ester **109**; however by Michaelis-Becker synthesis[105] the commercially available triflouroethylphosphite **107** was directly converted to the ester **109**. The ester upon Horner-Emmons reaction under Still-Gennari conditions[106] afforded the ester **115** giving only a 80:20 *Z*/*E* mixture.

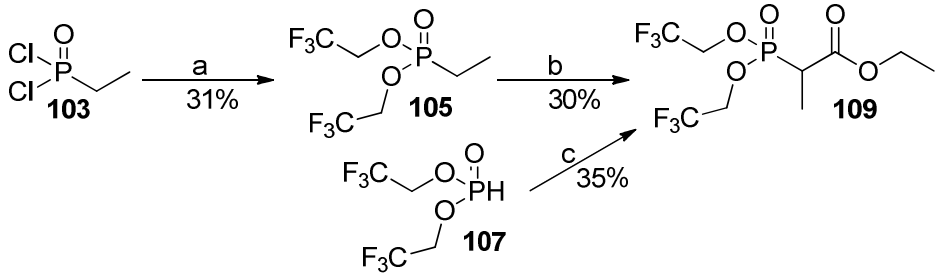


Figure 55: Reaction scheme for the synthesis of triflouroethyl-phosphonoate ester **109**[105,107]

Reactions and conditions: a) THF, 0 °C, Et_3N, bis(2,2,2)-triflouroethanol (**104**), rt, 2 h 20 min.; b) LiHMDS, THF, -78 °C, ethyl choroformate **106**, -20 °C overnight; c) NaH, THF, HMPA, ethyl 2-bromopropionate **108**, rt, 5 h.

Because this was not sufficiently stereoselective, the method of Mori et al. as shown in Figure 56 was tried. The required reagent was synthesized by treating phosphonate **110** with phosphorous pentachloride at 75-80 °C overnight to afford phosphoryl chloride **112**. *o*-Cresol **113** was esterified with **112** in the presence of triethylamine to afford the desired *tolyl*-phophonate ester **114**. The aldehyde **41** upon Wittig Horner-Wadsworth-Emmons (HWE)-reaction with phosphono acetate **114** in the presence of NaI and DBU afforded α,β-unsaturated-α-methyl ethyl ester **115** in 84% yield with 99:1 *Z*/*E* mixture.[108]

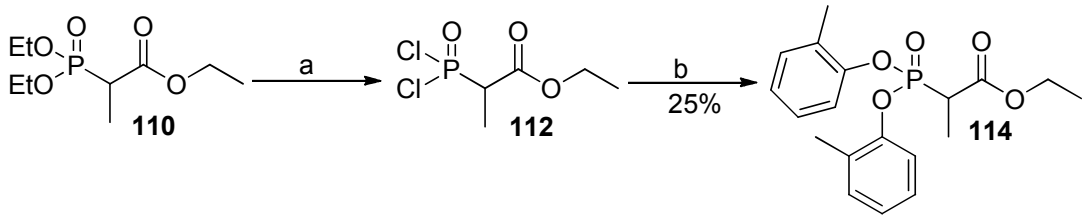


Figure 56: Reaction scheme for the synthesis of *tolyl*-phosphonoate ester **114**[109]

Reactions and conditions: a) benzene, 0 °C, PCl_5 **111**, 75 °C, 10 h; b) benzene, 0 °C, Et_3N, *o*-cresol **113**, rt, 1h.

The ester upon reduction with lithium aluminium hydride in diethyl ether afforded the allylic alcohol **116** in excellent yield. The allylic alcohol **116** upon sharpless asymmetric epoxidation[110] with Ti(O*i*-Pr)$_4$ in the presence of *t*-BuOOH and (-)-DET afforded the epoxide **117** in 70% yield with 96% *ee*. Opening of the epoxide and simultaneous protection of the hydroxyl group as triethyl silyl group and oxidation of the alcohol to aldehyde **119** in a one pot reaction was carried out with TESOTf **118** at -42 °C in dichloromethane and molecular sieves.[111] The aldehyde was synthesized in a micro scale reaction confirming the formation of the product through GC-MS analysis which needs to be scaled up for further analysis, as well as to forward the synthetic plan towards the side chain.

The slight change in the synthetic scheme by switching over from *cis*-α,β-unsaturated-α-methyl ethyl ester **115** to the *trans*-ester **124** was carried out as shown in the Figure 57.

The aldehyde upon Wittig reaction with phosphonium ylide **123** afforded *trans*-α,β-unsaturated-α-methyl ethyl ester **124** in 90% yield exclusively without any traces of the *cis* isomer.[112,113] The *trans*-ester upon reduction with Lithium aluminium hydride afforded the alcohol **125** in 87% yield. Sharpless asymmetric epoxidation[110] of the allylic alcohol afforded the epoxide **126** in 65% yield with 98% *ee*. Opening of the epoxide **126** with palladium catalyst **127** to **128** was not successful. Therefore, the hydroxyl group of the epoxide was protected with *tert*-butyldimethylsilyl group (**129)** to afford **130**.[114] This time the opening of the epoxide carried out again with the palladium catalyst afforded the product **131** in micro scale in mg amounts which needs to be scaled up for further analysis and to forward the synthesis toward the side chain.[115]

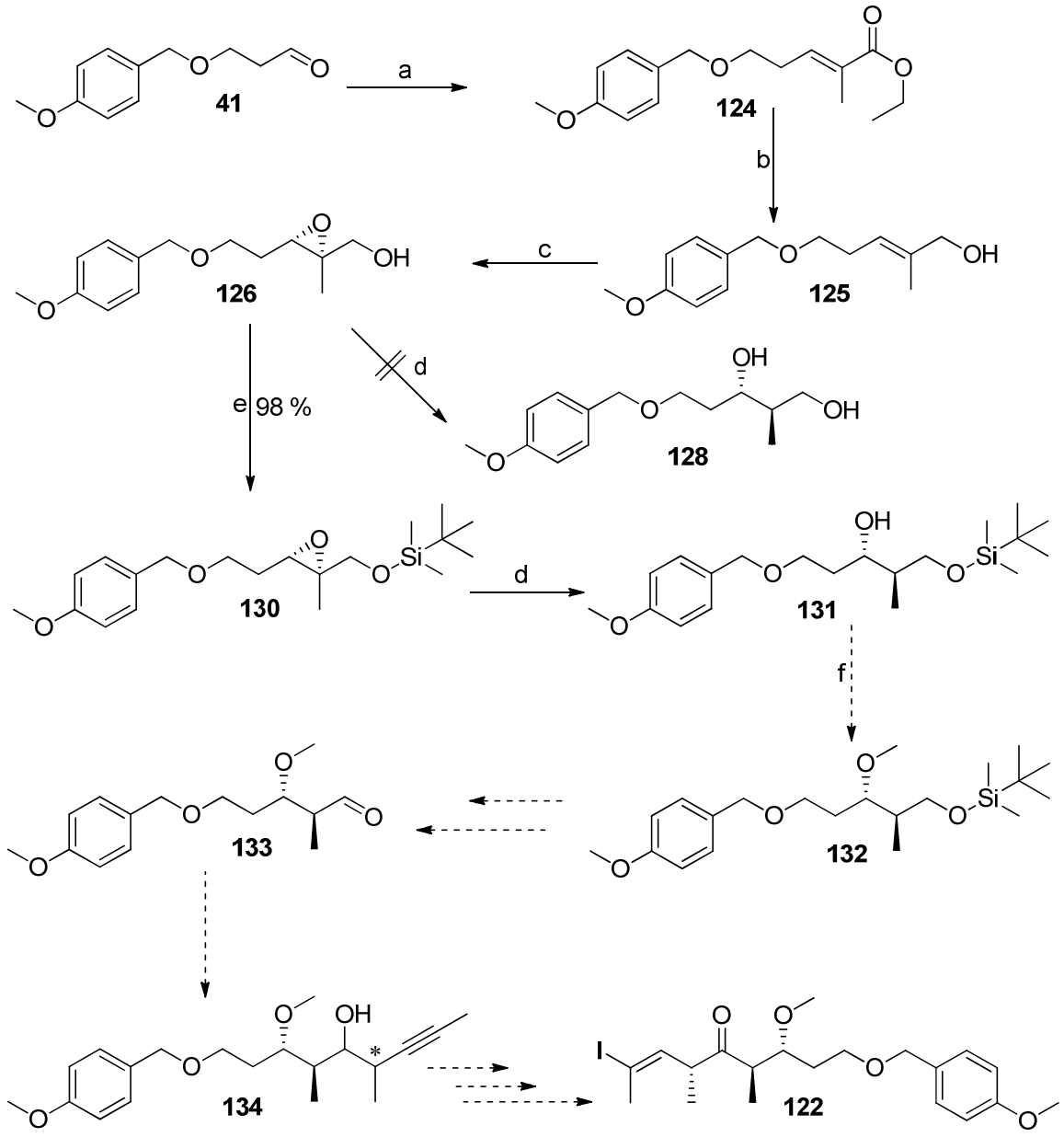
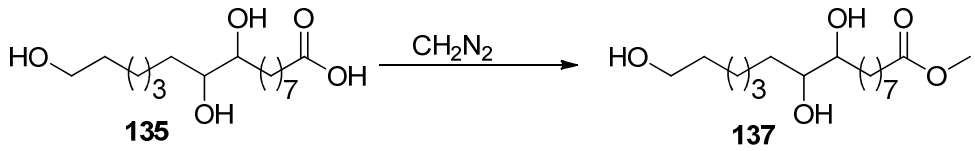


Figure 57: Reaction scheme of epoxide pathway via *trans* α,β-unsaturated α-methyl ethyl ester **124**

Reactions and conditions: a) CH_2Cl_2, $Ph_3P{=}C(CH_3)CO_2Et$ **123**, rt, 34 h; b) LAH, Et_2O, 0 °C, 15 min., Na_2SO_4, 0 °C, 15 min.; c) DCM, 4 A° mol. sieves, $Ti(O\textit{i}Pr)_4$, L-(+)-DET, -30 °C, 30 min., TBHP, -20 °C, 3 h; d) $Pd(dba)_3CHCl_3$ **127**, 1,4-dioxane, *n*-Bu_3P, HCOOH, Et_3N, rt, 13 h, e) CH_2Cl_2, imidazole, TBDMSCl **129**, rt, 3 h; f) Proton sponge, CH_2Cl_2, MeO_3BF_4, rt.

4 Role of the carboxylic moiety in carolacton activity

Carolacton is a carboxylic acid and active at low pH. To address the question whether the activity depends on ion dissociation of the acid function, a methyl ester derivative **138** was needed. It is important to know whether the carboxylic acid group of carolacton is needed for activity. Therefore, the esterification of the acid group was investigated. A difficulty was that only few mg of carolacton were available. Therefore, aleuritic acid was used as model compound for the esterification process as well as for the analysis of the reaction products by HPLC/MS. Aleuritic acid contains some of the characteristic structural elements of carolacton, the diol group and the terminal acid function. In order to establish the carolacton methyl ester first the model compound aleuritic acid **135** (mol wt: 304.43) was first analyzed with HPLC/MS by preparing its methyl ester **137** (mol wt: 318.43) in micro scale apparatus (Figure 59) in mg amounts with diazomethane **136**. The apparatus and the synthetic procedure utilized in the synthesis of carolacton methyl ester and aleuritc acid was much the same conditions as depicted below in the Figure 58.



135 **137**

Figure 58: Conversion of aleuritic acid **135** to its methyl ester derivative **137**.

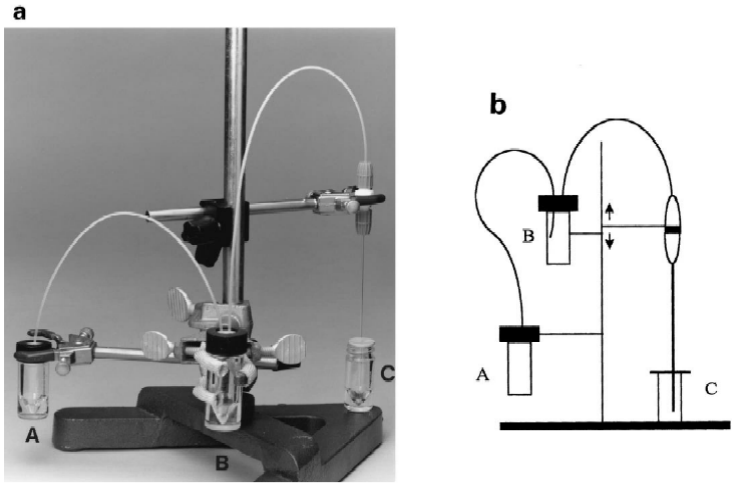


Figure 59: Apparatus for the generation of the diazomethane **136** for methylation.[116] **a**: Reaction vials mounted on a tripoid stand A:Reactor vial, B: trap and C: collector vial. **b**: Line diagram of the described set-up.

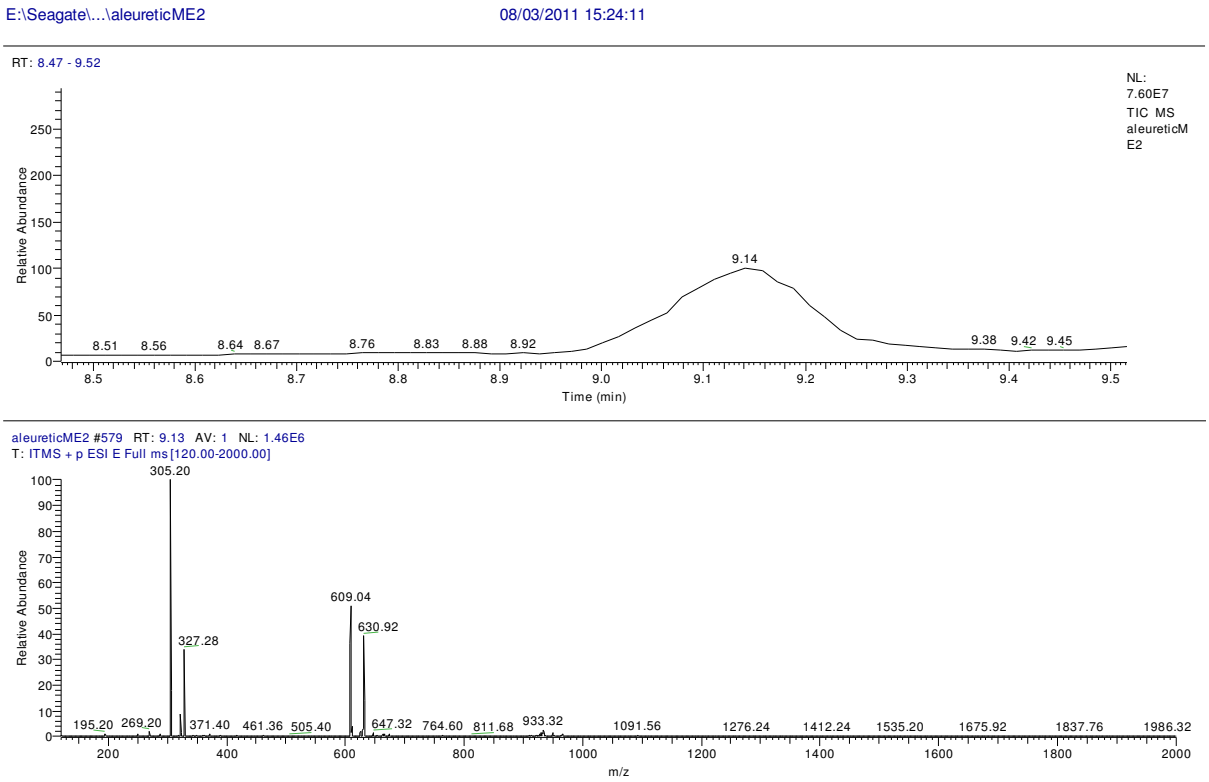


Figure 60: HPLC-Chromatogram of aleuritic acid **135** at R_t: 9.14 min. and its mass spectrum.

The HPLC-Chromatogram (Figure 60) using electro spray ionization (ESI) in positive ionization mode at R_t 9.14 min. shows the characteristic mass ions of of **135** at *m/z* = 305.20 $[M+H]^+$, 327.28 $[M+Na]^+$, 327.43 $[M+Na]^+$, 609.04 $[2M+H]^+$, 630.92 $[2M+Na]^+$.

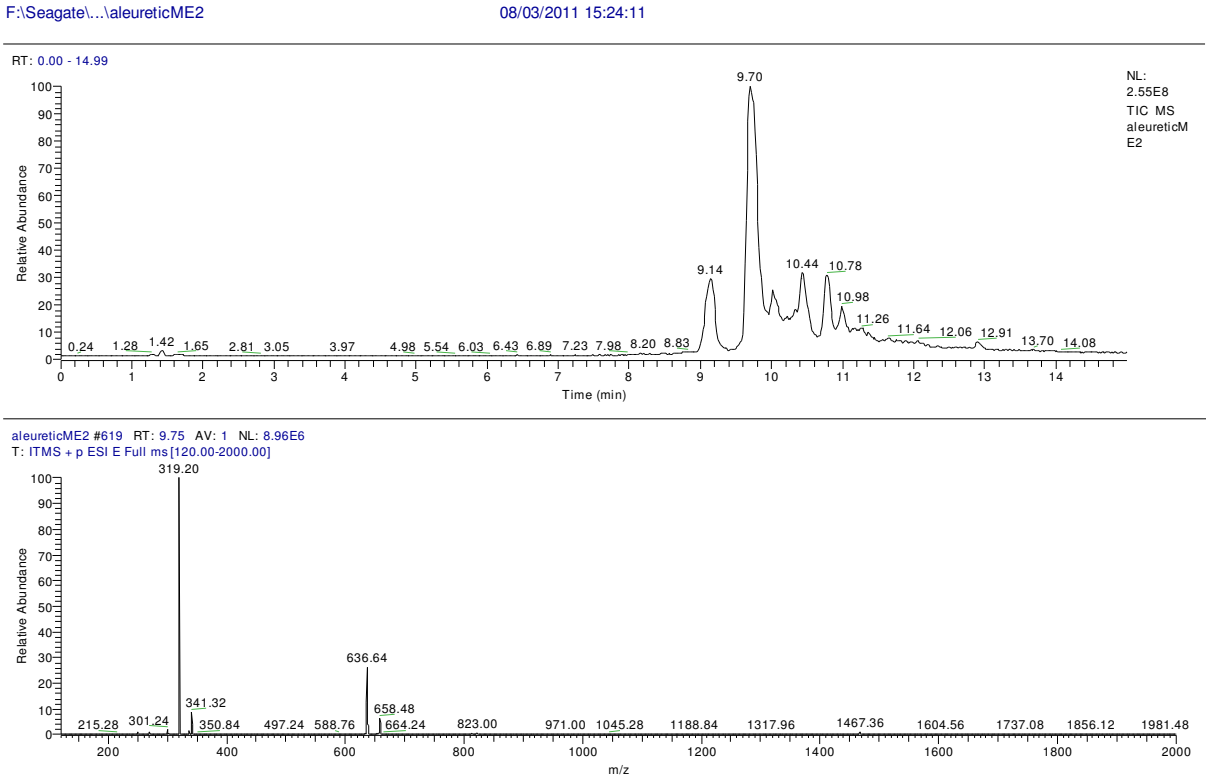


Figure 61: HPLC-Chromatogram of aleuritic acid methyl ester **137** at R_t: 9.70 min. and its mass spectrum.

The HPLC-Chromatogram (Figure 61) using electro spray ionization (ESI) in positive ionization mode at R_t 9.70 min. shows the characteristic mass ions of of **137** at *m/z* = 319.20 $[M+H]^+$, 341.32 $[M+Na]^+$, 636.64 $[2M]^+$, 658.48 $[2M+Na-H]^+$.

The methyl ester of carolacton **138** and carolacton **13** containing free carboxylic acid function were again tested for the effect on the biofilm damage of *S.mutans* as represented in Figure 62 B by the group of Wagner Döbler et.al.[43] The methyl ester of carolacton was tested in three different concentrations for its biological activity against *S.mutans* biofilms using LIVE/DEAD viability staining.

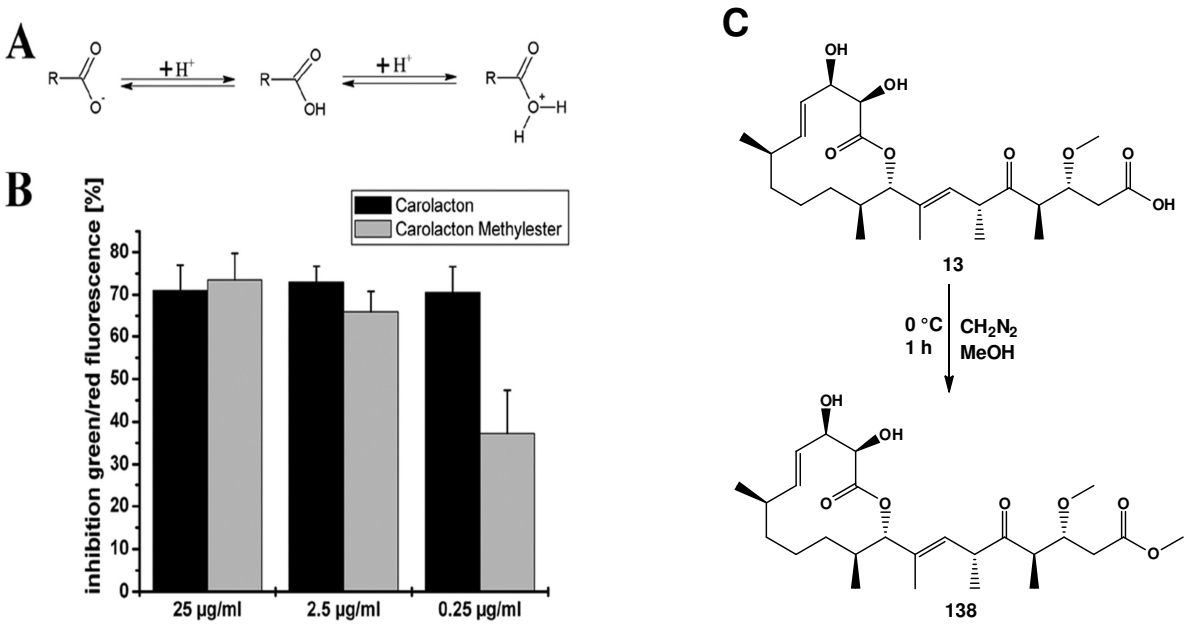


Figure 62: Influence of the carboxylic moiety on carolacton activity.[43]

A) Change of the charge of the carboxylic acid function of carolacton upon decreasing pH.
B) Inhibition of biofilm viability by carolacton and the carolacton methylester for 3 different inhibitor concentrations determined by LIVE/DEAD staining.
C) Conversion of carolacton into the corresponding carolacton methylester.

Following the methylation procedure of aleuritic acid **135** to its methyl ester **137** (Figure 57), carolacton **13** (1 mg, mol.wt: 468.58) was utilized in this synthesis (Figure 62 C) with excess of diazomethane **136** for 1 h at 0 °C to generate the carolacton methyl ester **138** (mol. wt: 482.58). Figure 62 shows that the inhibition of viability caused by carolacton methyl ester was the same as the natural carolacton molecule for concentrations 2.5 and 25 µg/mL. However for the lowest tested concentration of 0.025 µg/mL, the inhibition was slightly reduced to 35%. Hence it is assumed that no functional activation of the molecule at low pH occurs and carolacton is independent of its pH. By considering the above results it is evident that carboxylic group of carolacton doesn't play a dominant role in its inhibitory activity.

Recently, new results contradicted this report. The methyl ester **138** is easily cleaved by the bacteria to carolacton **13** (I. Wagner-Döbler, pers. commun.). Therefore, the acid function in carolacton **13** seems to be necessary.

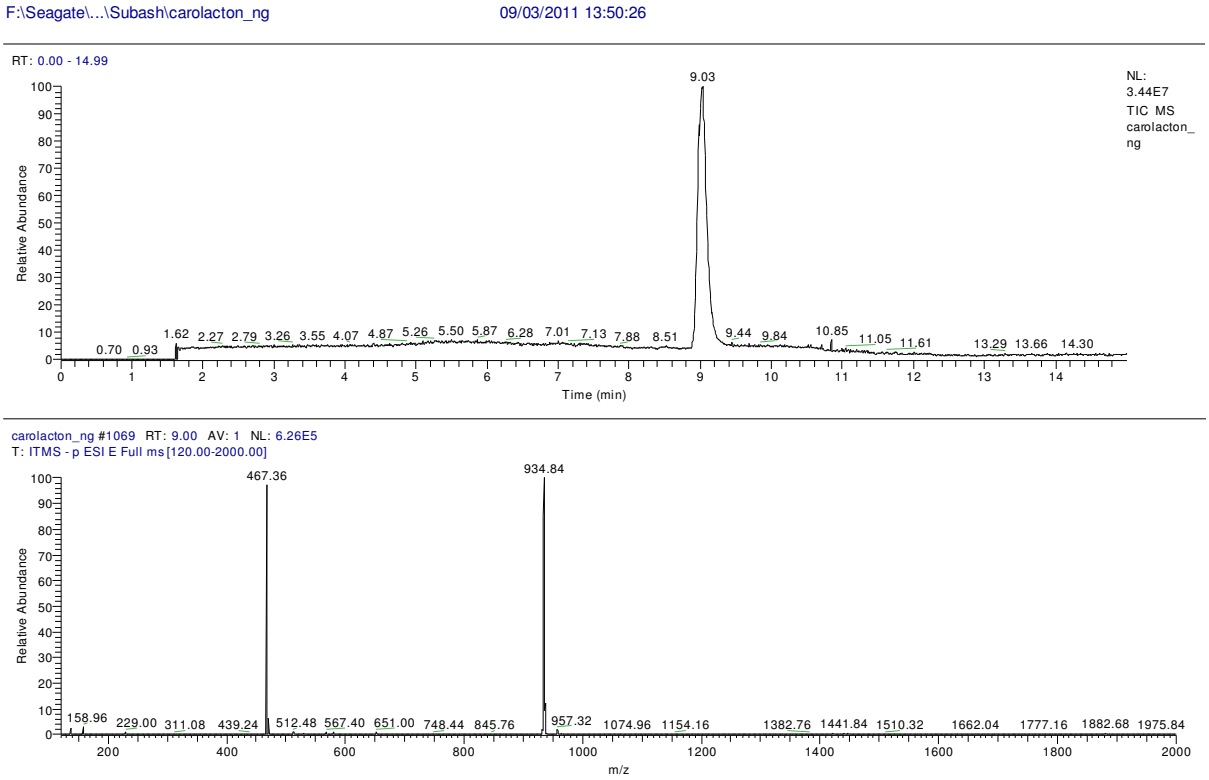


Figure 63: chromatogram of carolacton **13** at R_t: 9.03 min. and its mass spectrum.

The HPLC-Chromatogram of **13** (Figure 63) using electro spray ionization (ESI) in negative ionization mode at R_t 9.03 min. shows the characteristic mass ions of **13** at *m/z* = 467.36 $[M-H]^-$, 512.48 $[M+2Na-2H]^-$, 934.84 $[2M-2H]^-$, 957.32 $[2M+2Na-2H]^-$.

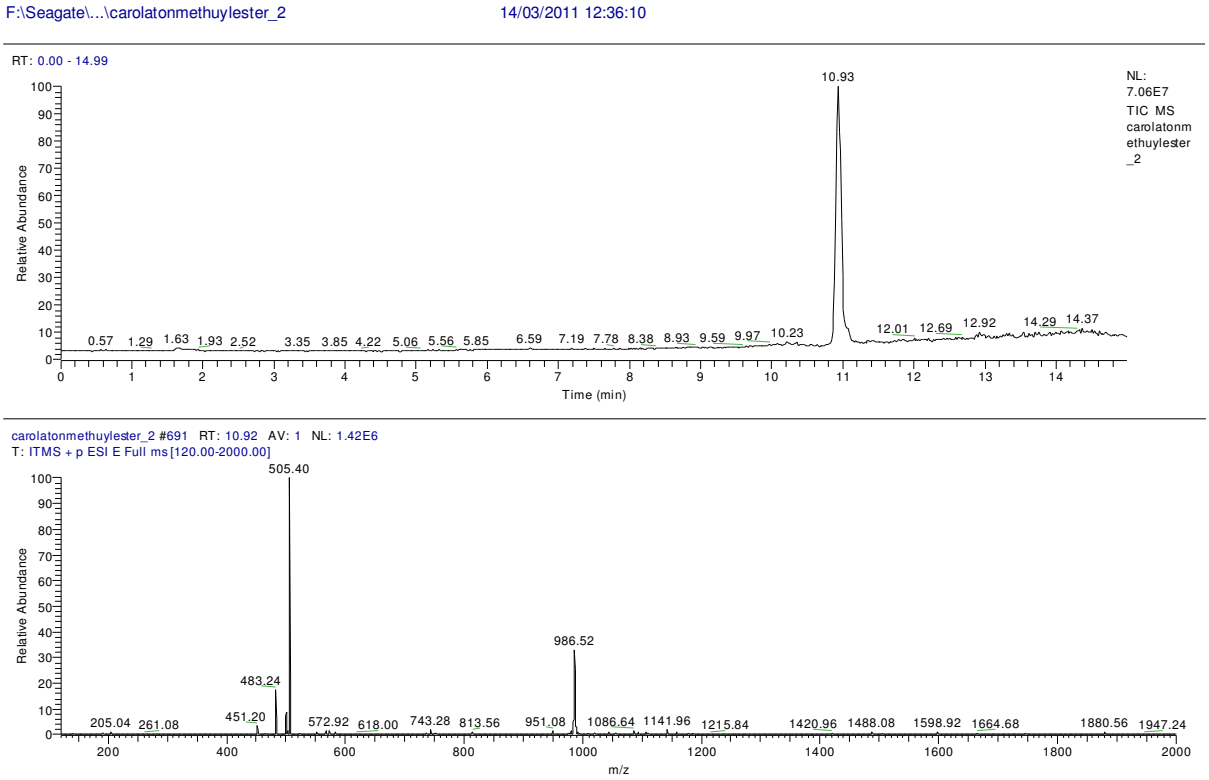


Figure 64: chromatogram of carolacton methyl ester **138** at R_t: 10.93 min. and its mass spectrum.

The HPLC-Chromatogram of **138** (Figure 64) using electro spray ionization (ESI) in positive ionization mode at R_t 10.92 min. shows the characteristic mass ions of of **138** at *m/z* = 483.24 $[M+H]^+$, 505.40 $[M+Na]^+$, 986.52 $[2M+Na-H]^+$.

In the previous studies towards the effectivity of carolacton it was concluded that the nature of the growth of cells play a major role along with the initial pH value of the relevant culture. Also the pH of the biofilm falls significantly faster than that of the planktonic cell cultures. The effect of carolacton on biofilms at pH above 5 has no significant effect while the addition of carolacton to the biofilms at pH below 5 leads to the significant damage of biofilms of *S. mutans*.

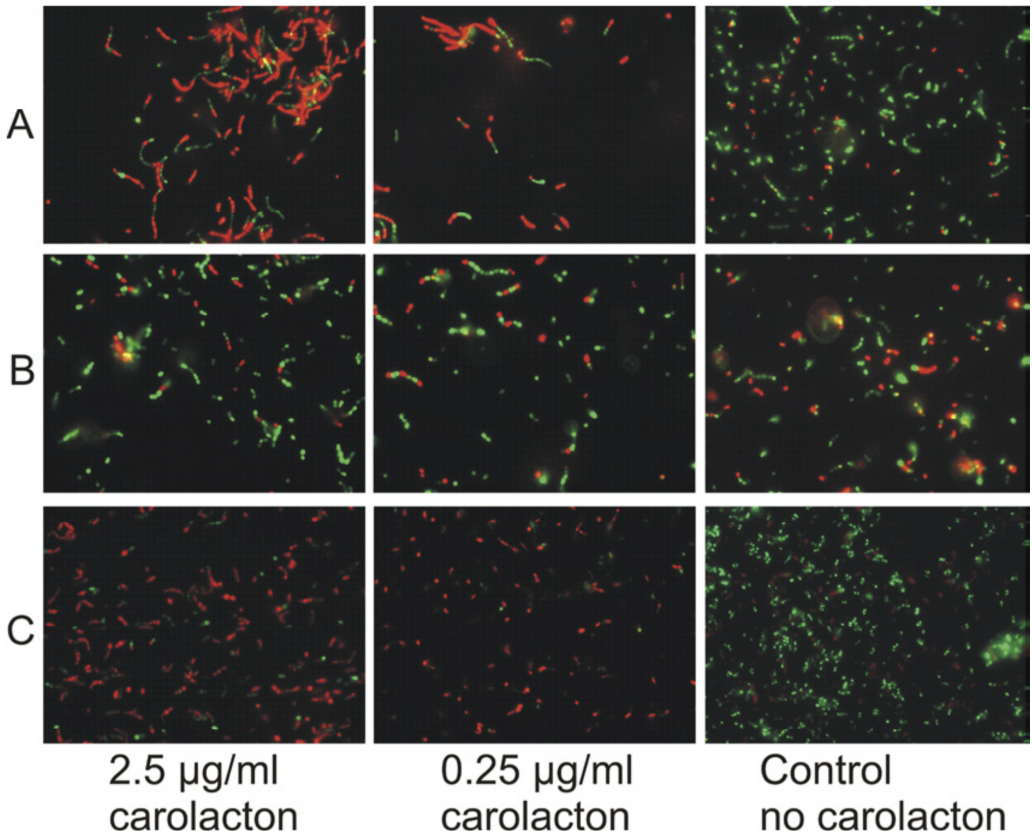


Figure 65: Verification of the insensitivity of a pknB-deficient strain to carolacton using LIVE/DEAD staining and fluorescence microscopy. Images of carolacton-treated and untreated biofilm cells of the wild type (A), the Δ*pknB* mutant (B), and the complemented strain (C).[43]

The most important discovery of the working group was however the identification of the gene which is responsible for the activity of carolacton. Two-component systems (TCS), serine-threonine protein kinases (STPKs) and phosphatases were used by bacteria to detect, transmit and react to the signals by the outside world. Most of the bacterial STPKs were predicted to consist of an N-terminal kinase domain, and a C-terminal sensory domain located extracellularly. TCS widely occurred in prokaryotes; consist of a membrane-located sensor histidine kinase and a correspondence response regulator present in the cytoplasm. In response to an environmental signal, the histidine kinase (HK) protein is autophosphorylated on a histidine residue and in the later stage regulates the transcription of target genes. Similar to histidine kinases, STPKs are autophosphorylated, but on the serine or threonine residues. In contrast to two-component response regulators, STPKs exert their effect by phosphorylation of target proteins. *Streptococcus mutans* contains one serine-threonine kinase, encoded by *pknB*. It was found that the *pknB* knockout mutant is insensitive to carolacton while the wild type and complementary strain shown to be effective upon treatment with carolacton as shown in the Figure 65.

Furthermore, the stability of the carolacton along with the standard reference aleuritic acid **135** in water and different buffer solutions was tested. The samples were prepared (100 µg of **135** and carolacton **13** per 1mL buffer and unbuffered solutions) with buffer and unbuffered solutions. The samples were stored at room temperature and after 4 months analyzed with HPLC/MS. The results are presented in Figure (66-71). By analysis of the mass spectras of the HPLC-Chromatogram in all different media listed below it was clear that carolacton is quite stable in these solutions, without any degradation. The reference for the HPLC-Chromatogram of **13** is shown in Figure 63, using electro spray ionization (ESI) in negative ionization mode. The different solutions used are mentioned below. The analysis was performed under the same conditions as reported in Figure 58:

1) Carolacton in water

2) PBS (Phosphate Buffered Saline solution)

3) THB (Todd Hewitt Broth unbuffered solution)

4) THBS (Todd Hewitt Broth with 1% sucrose unbuffered solution)

5) THB + Buffer solution pH 6.5 (75 mM Phosphate buffer Na_2HPO_4 and NaH_2PO_4)

6) THB + Buffer solution pH 7.8 (75 mM Phosphate buffer Na_2HPO_4 and NaH_2PO_4)

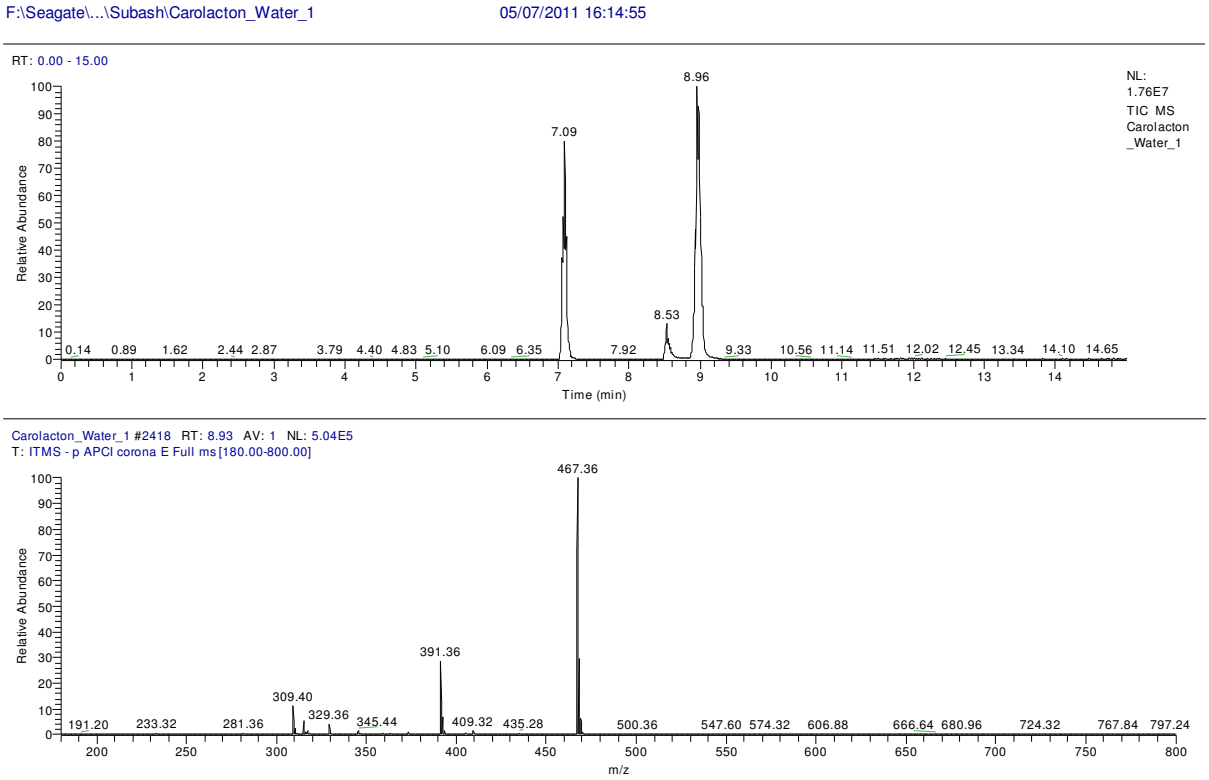


Figure 66: HPLC-Chromatogram of carolacton **13** in water after 4 months.

The HPLC-Chromatogram (Figure 66) using atmospheric chemical ionization (APCI) in negative ionization mode shows two peaks at R_t 7.09 min. and 8.96 min. representing the characteristic mass ion of aleuritic acid **135** which was used as internal standard at *m/z* = 303.24 $[M-H]^-$ and carolacton **13** at *m/z* = 467.36 $[M-H]^-$ respectively.

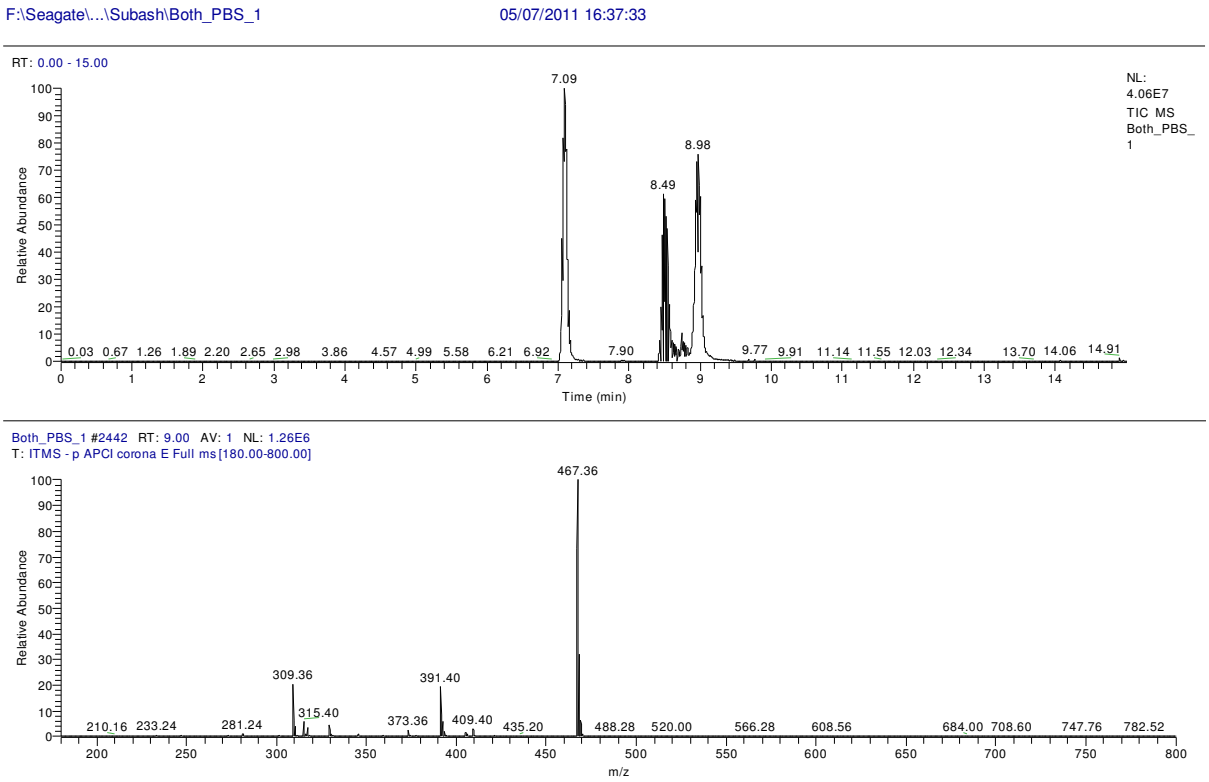


Figure 67: HPLC-Chromatogram of carolacton **13** in PBS after 4 months.

Ingredients in PBS (Phosphate Buffered Saline):

137 mM NaCl

2.7 mM KCl

12 mM Phosphate

The HPLC-Chromatogram (Figure 67) using atmospheric chemical ionization (APCI) in negative ionization mode shows the peak at R_t 7.09 min. representing the characteristic mass ion of **135** at *m/z* = 303.24 $[M-H]^-$, and the other two peaks at 8.49 min. and 8.98 min. having the characteristic mass ion of **13** at *m/z* = 467.36 $[M-H]^-$.

Under these conditions it was observed the epimerization seems to take place at R_t 8.49 min. with the halflife ($t_{1/2}$) of approx. 120 days. The degree of epimerization of **13** in water (Figure 66) seems to be very less compared to the chromatogram in PBS. The ratio of the epimerized carolacton and carolacton in PBS was 1.0:1.27.

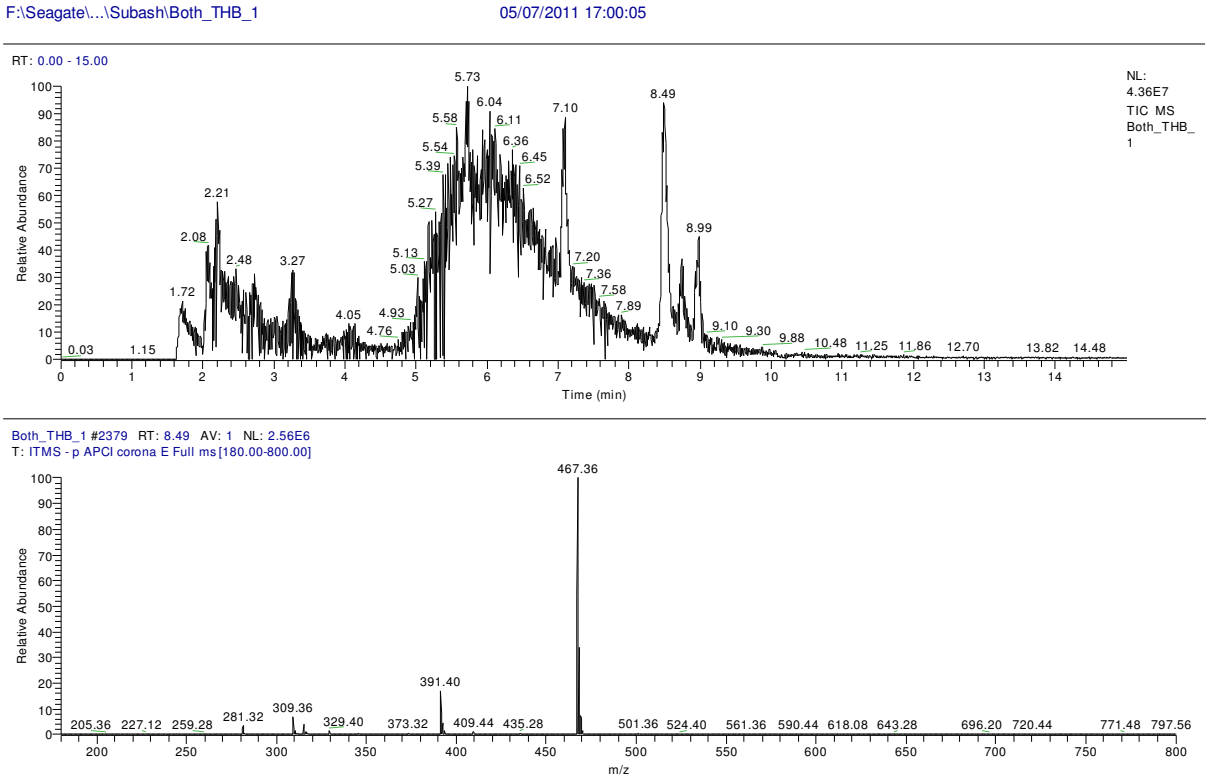


Figure 68: HPLC-Chromatogram of carolacton **13** in THB unbuffered solution after 4 months.

Ingredients in THB (Todd Hewitt Broth unbuffered solution):

Heart Infusion 3.1 g/L

Neopeptone 20 g/L

Dextrose 2 g/L

NaCl 2 g/L

NaH_2PO_4 0.4 g/L

$NaHCO_3$ 2.5 g/L

The HPLC-Chromatogram (Figure 68) using atmospheric chemical ionization (APCI) in negative ionization mode shows the peak at R_t 7.10 min. representing the characteristic mass ion of **135** at *m/z* = 303.24 $[M-H]^-$, and the other two peaks at 8.49 min. and 8.99 min. having the characteristic mass ion of **13** at *m/z* = 467.36 $[M-H]^-$.

The ratio of the epimerized carolacton and carolacton in THB was 2.6:1.0 compared to the chromatogram of **13** in PBS having the ratio of 1.0:1.27. There was a drastic increase in the epimerization of carolacton in THB compared to the degree of epimeriazation of carolacton in PBS (Figure 67).

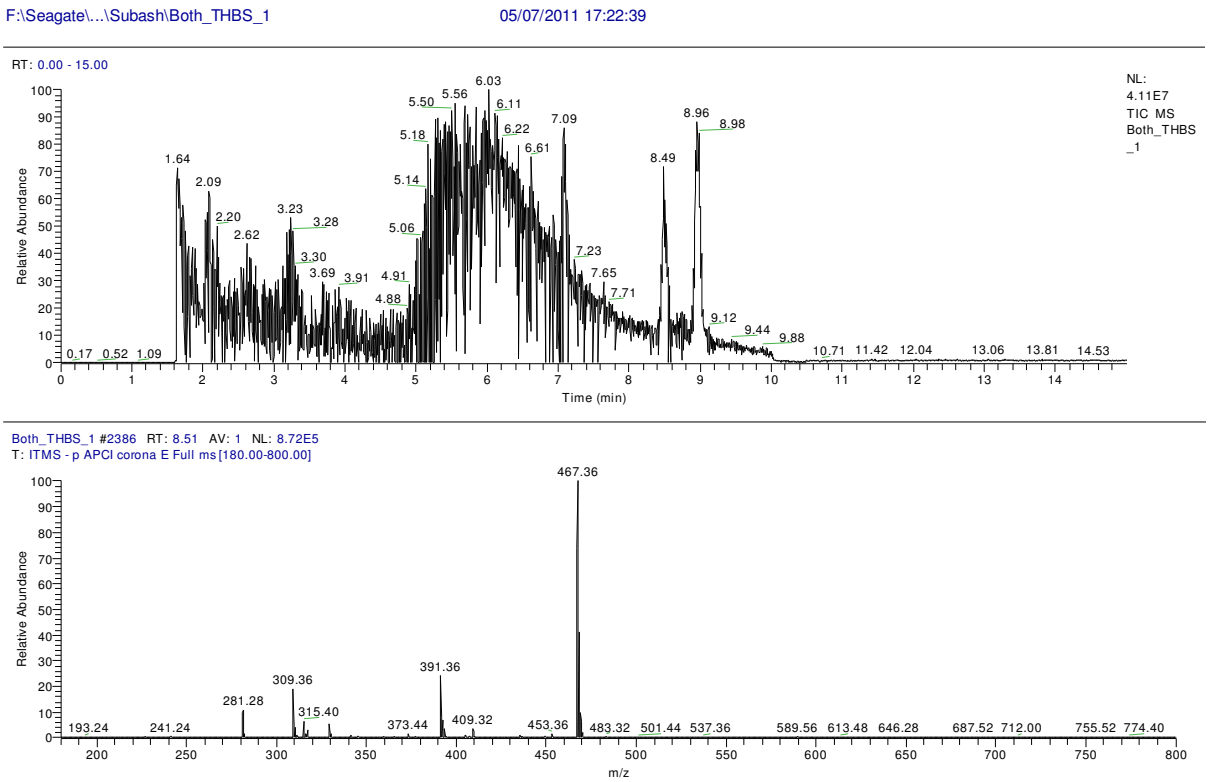


Figure 69: HPLC-Chromatogram of carolacton **13** in THBS unbuffered solution after 4 months.

Ingredients in THBS (Todd Hewitt Broth in 0.5% sucrose unbuffered solution):

Heart Infusion 3.1 g/L

Neopeptone 20 g/L

Dextrose 2 g/L

NaCl 2 g/L

NaH_2PO_4 0.4 g/L

$NaHCO_3$ 2.5 g/L

The HPLC-Chromatogram (Figure 69) using atmospheric chemical ionization (APCI) in negative ionization mode shows the peak at R_t 7.09 min. representing the characteristic mass ion of **135** at *m/z* = 303.24 $[M-H]^-$, and the other two peaks at 8.49 min. and 8.96 min. having the characteristic mass ion of **13** at *m/z* = 467.36 $[M-H]^-$.

The ratio of the epimerized carolacton and carolacton in THBS was 1.0:1.22. The degree of epimerization was slightly higher with marginal increase of epimerized carolacton compared in PBS of ratio 1.0:1.27(Figure 67).

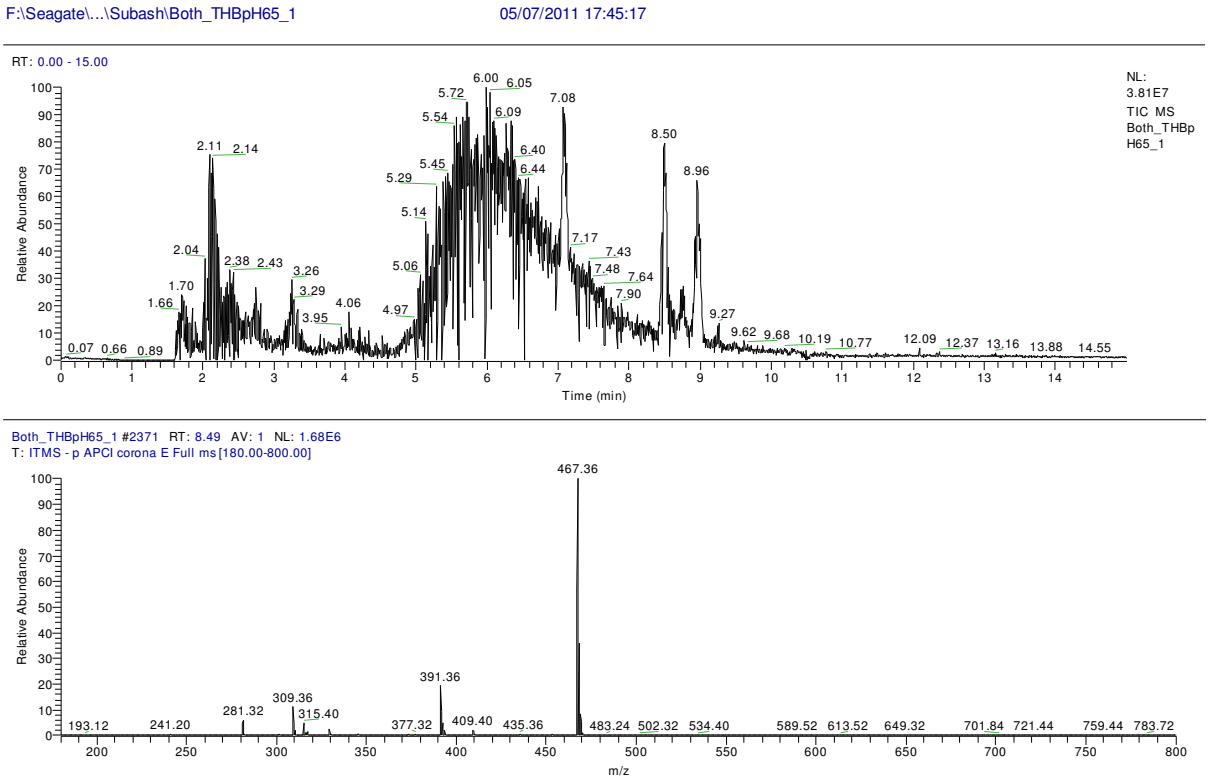


Figure 70: HPLC-Chromatogram of carolacton **13** in THB buffered solution pH 6.5 after 4 months.

The HPLC-Chromatogram (Figure 70) using atmospheric chemical ionization (APCI) in negative ionization mode shows the peak at R_t 7.08 min. representing the characteristic mass ion of **135** at *m/z* = 303.24 $[M-H]^-$, and the other two peaks at 8.49 min. and 8.98 min. having the characteristic mass ion of **13** at *m/z* = 467.36 $[M-H]^-$.

The ratio of the epimerized carolacton to the carolacton in THB buffered solution pH6.5 is approx. 1.30:1.0 compared to the chromatogram having the ratio 2.6:1.0 n in THB unbuffered solution (Figure 68). The rate of epimerization was more in the THB unbuffered solution compared to the buffered solution of pH 6.5.

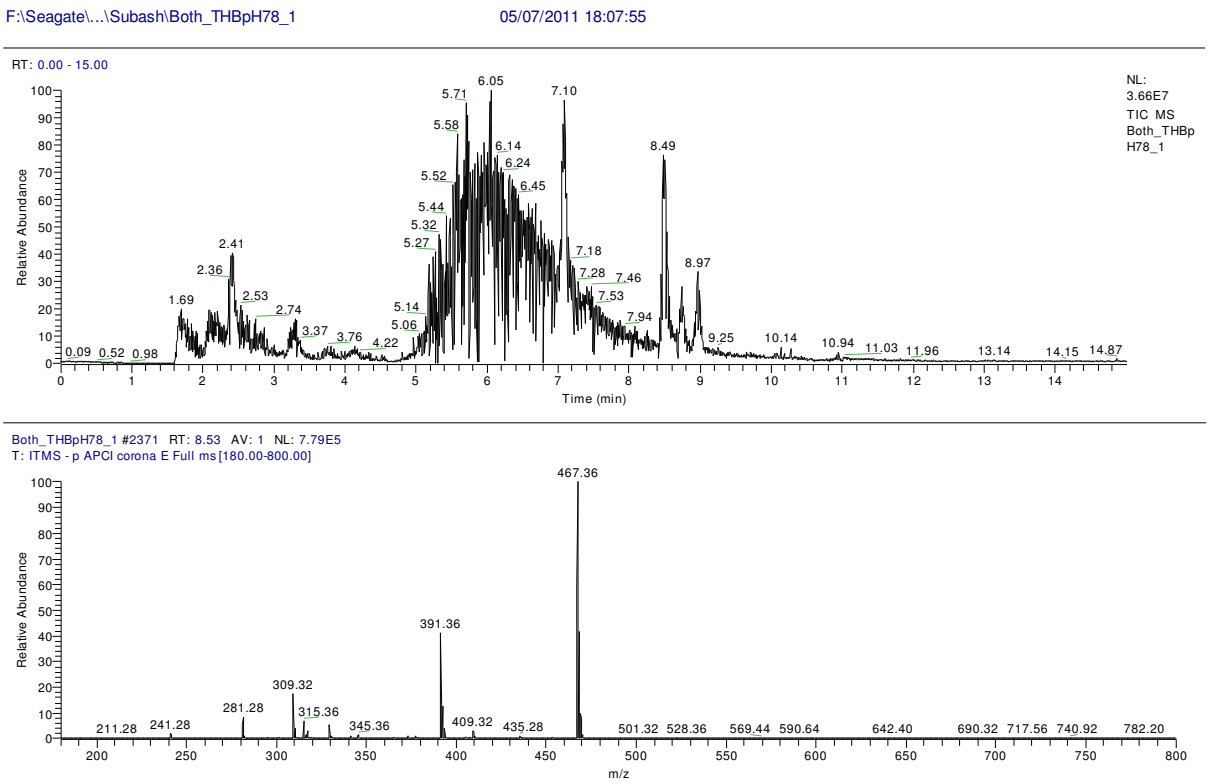


Figure 71: HPLC-Chromatogram of carolacton **13** in THB buffered solution pH 7.8 after 4 months.

The HPLC-Chromatogram (Figure 71) using atmospheric chemical ionization (APCI) in negative ionization mode shows the peak at R_t 7.10 min. representing the characteristic mass ion of **135** at *m/z* = 303.24 $[M-H]^-$, and the other two peaks at 8.49 min. and 8.97 min. having the characteristic mass ion of **13** at *m/z* = 467.36 $[M-H]^-$.

Compared to the previous case the ratio of the epimerized carolacton to the carolacton in THB buffered solution of pH 7.8 was more enhanced 2.7:1.0. To sum up, the the rate of epimerization was more in the THB buffered solution of pH 7.8 and THB unbuffered solution compared to the THB buffered solution of pH 6.5 in a decreasing order.

5 Summary and Outlook

The attempts to synthesize the carolacton side chain was discussed in the above chapter **3** and the idea of synthesizing the carolacton side chain in using the above synthetic procedures was to synthesize various derivatives of carolacton by altering the functional groups with out deviating from the proposed synthetic reactions.

Aldol reactions play a prominent role in securing the right stereochemistry of the side chain of carolacton. The synthesis was initiated with proline catalyzed enantioselective aldol reaction but it was later clear that there is the necessity of usage of the various ligands/substituents on the proline catalyst to achieve good enantioselectivities. Therefore, it was not explored further and new strategies developed for the synthesis. During the synthesis of the side chain with the relative configured sterechemistry in the chapter **3.1** (Figure 28) optimization of reaction conditions were carried out in generating the methylated product and the best optimum yield and the etherification of the hydroxyl group was successfully carried out by using meerwein salt as methylating agent. The retrosynthetic approach towards the side chain was show in the Figure 72.

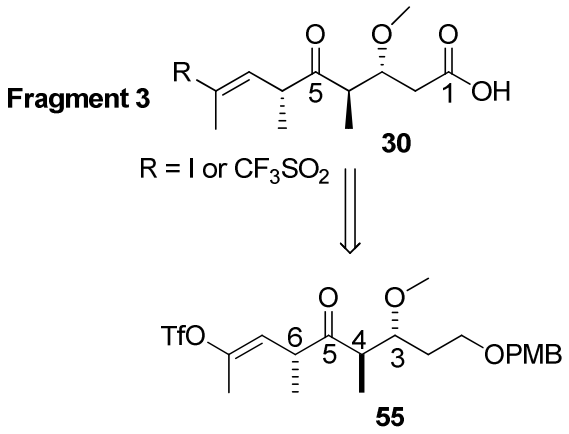


Figure 72: Retrosynthetic approach towards the carolacton sidechain

Finally, after generation of the diketone **53** based on the relative stereochemistry the synthesis of the side chain **30** with absolute configuration was carried out by synthesizing the chiral ketones with terminal alkene and alkyne groups using Evans auxialaries and SAMP methodology as discussed in chapter **3.2**. The problems associated in the synthesis of the chiral ketone with internal alkyne were minimized by isomerizing the acid of terminal alkyne with KO*t*Bu and DMSO at room temperature. However, the best yields were obtained with the ketones containinig terminal alkene instead of the terminal or internal alkynes. The

Evans methodology for generating the terminal alkene was preferred over the SAMP methodology due to its excellent enantioselectivities of chiral ketones. Various chiral ketones synthesized were shown in the Figure 73.

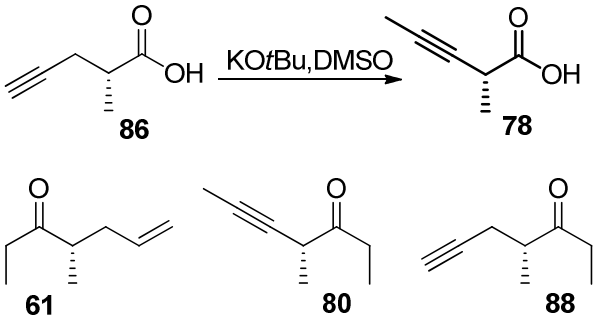


Figure 73: Synthesized chiral ketones internal alkene **61** and internal and terminal alkynes **80** and **88**.

The determination of the absolute configuration with MTPA esters **97** and **98** in this thesis chapter **3.3.1** required to analyze the 2D NMR data and the difference in the chemical shifts $\Delta\delta^{RS}$ values of the diastereomers were very close; musch easier method would be utilizing the α-methoxy-α-phenylacetic acid (MPA) as chiral derivatizing agents (CDA) (Figure 74), instead of the MTPA leading to assign the chemical shifts (δ) of either diasereomers of MPA ester **139** in a much uncomplicated way to give higher $\Delta\delta^{RS}$ values that are more reliable and homogeneous in terms of distribution of the signs. The reason that MPA as CDA is much simpler than MTPA was due to the presence of three main conformers with similar populations. Also some of these conformers produce shielding effects and others deshielding effects on substituents L_1/L_2, resulting in the small $\Delta\delta^{RS}$ values and sometimes abnormalities in the distribution of signs. In contrast to the above MTPA esters **140** (in general usage for different L_1 and L_2's), MPA esters **139** have only two conformers and clear preference for one of these two conformers. This results in transmitting the shielding effect to the substituents to give high $\Delta\delta^{SR}$ values, giving the edge for the MPA esters over the MTPA esters.[117]

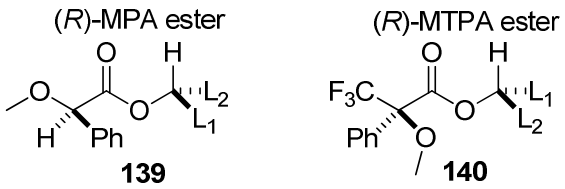


Figure 74: Esters **139** and **140** of the aldol product with different CDA's

Various protecting groups were employed in the synthesis of protected Hydroxy propanal. The para-methoxy benzyl groups is the best out of them becase it with stands reactions in various reaction conditions. The easy removal of the protecting group (OPMB) with DDQ at the end of the final synthesis of the carolacton is also an advantage. The asymmetric aldol reaction can be carried out with a chiral ketone with an achiral aldehyde under Paterson Aldol conditions to obtain the aldol product in good yield with 2:1 *anti*/*syn* diastereomeric ratio. The diastereomeric ratio (d.r.) can still be enhanced by optimizing the reaction conditions. Aldol reactions with terminal and internal alkynes in similar fashion as like that of a chiral terminal alkene could be carried out in order to observe any variations in the steroselectivity ratio of the isomers. The internal alkyne aldol reaction on reaction with Schwartz's reagent would provide vinyl iodide **122** and with terminal alkyne upon oxidation in the presence of $Hg(OAc)_2$ would provide the diketone similar to the case of the terminal alkene synthetic pathway.

In the chapter **3.4** as illustrated in the Figure 54 and 57 of the epoxide scheme the aldehydes **119** and **133** upon asymmetric allenyl boronation reported by Rousch et al.[118] leads to the internal alkyne product. Oxidation of these internal hydroxyl alkyne products **121** and **134** afford the corresponding ketones and transformation of the internal alkyne to vinyliodide furnishes the desired product with good stereoselectivity. The allene was synthesized from the propargylic alcohol in two steps as shown in Figure 75.

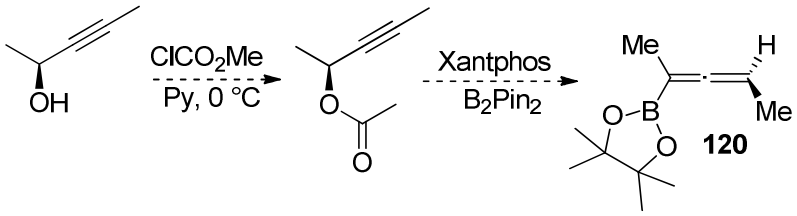


Figure 75: Proposed synthesis of boronated allene **120**

The aldehyde derived from **119** (Figure 54) and **133** (Figure 57) in the presence of phosphoric acid (***R***)-**141** and boronated allene **120** would give rise to the alcohol with 3,6-*syn* and 4,6-*anti* related configurations (Figure 76). Oxidation of the hydroxyl group at C-5 to ketone followed by the protection of ketone as dioxalane and finally utilizing Schwartz's reagent the desired molecule vinyl Iodide **122** can be synthesized.

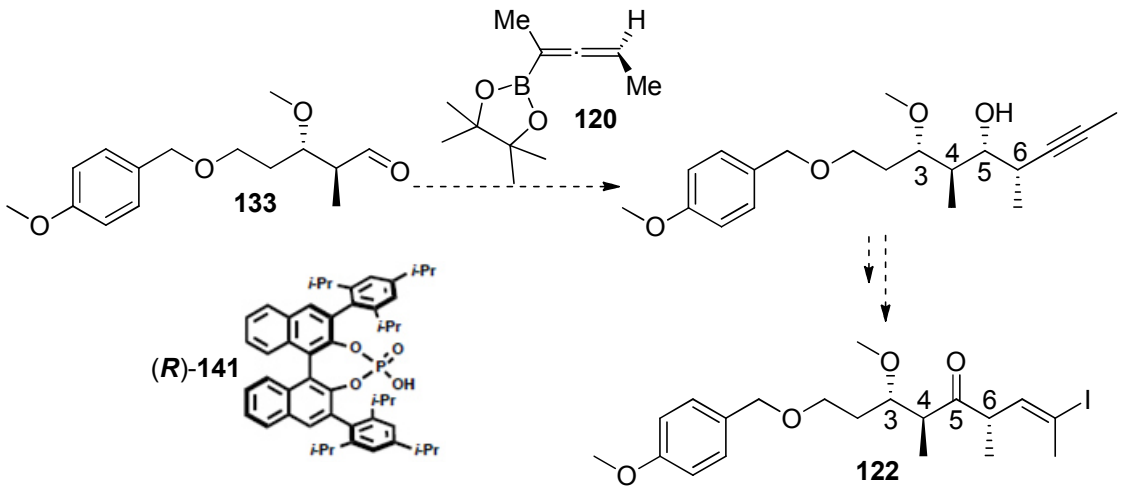


Figure 76: Proposed synthesis of the side chain of carolacton *via* non aldol process (epoxide pathway)

An other alternative pathway to the aldol reaction was the decarboxylative allylation reaction of the formed aldol product using the Evans auxiliary from propionyloxazolidinone and aldehyde to obtain the *anti*-configured aldol using Evans aldol reaction[119] followed by the cleavage of the auxiliary and generating the ketone with EtMgBr via Weinreb amide as shown in Figure 77 below.

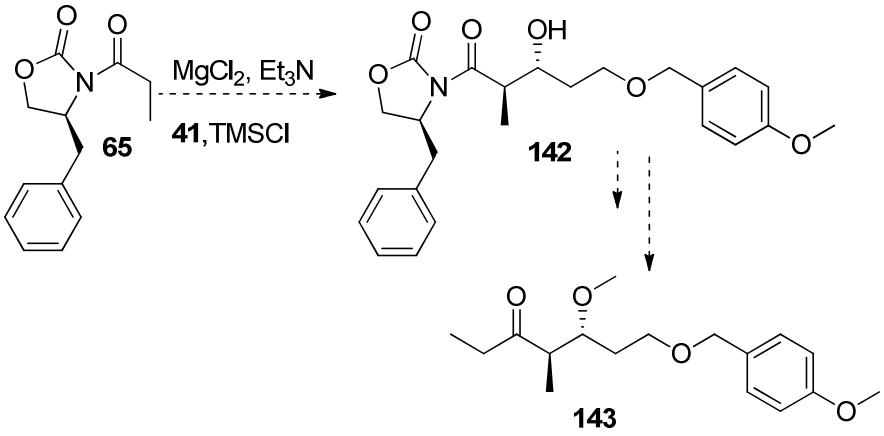


Figure 77: Proposed Evans aldol reaction with achiral aldehyde

Later by using the palladium-catalyzed decarboxylative asymmetric allylic alkylation (DAAA) reaction conditions developed by Trost et al.[120] a new stereogenic center with the allyl group adjacent to the keto group can be introduced to obtain **146**. The reaction proceeds through the (*Z*)-enol carbonate **145** as shown in the Figure 78.

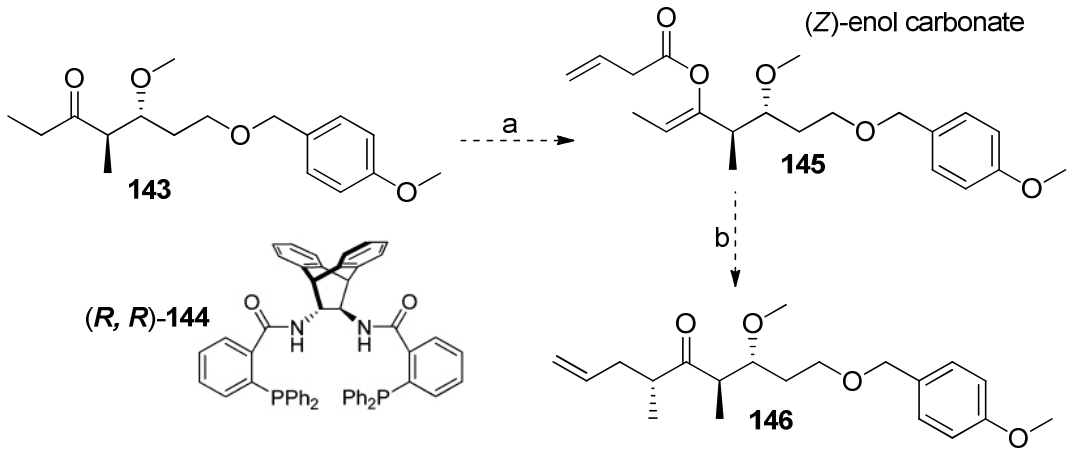


Figure 78: Proposed palladium-catalyzed decarboxylative asymmetric allylic alkylation (DAAA)

Reactions and conditions: a) $(PhMe_2Si)_2NLi$, THF, -78 °C, allyl chloroformate; b) $Pd_2(dba)_3CHCl_3$, Ligand (***R,R***)-**144**, dioxane, rt.

The role of the caroboxylic acid in the side chain of carolacton in the biological activity of carolacton was clearly known by synthesizing its methyl ester derivative which explains it's not the solely responsible factor for the activity of carolacton. Furthermore the stability of the carolacton in different buffered solutions of varied pH conditions was analyzed with HPLC/MS and it found to be stable in these solutions. Degedration of carolacton **13** was not observed in the HPLC-Chromatogram analysis, but under those conditions reported in the chapter **4** (Figure 66-71) slow epimerization seems to take with $t_{1/2}$ approx. 120 days. It would be much interesting to synthesize a wide variety of derivatives and analogues of carolacton as shown in the Figure 79 to know the role played by them in various biological activities. The synthetic scheme mentioned in this thesis is quite flexible in altering the functional groups and very feasible once the conditions are better optimized in the key aldol reaction.

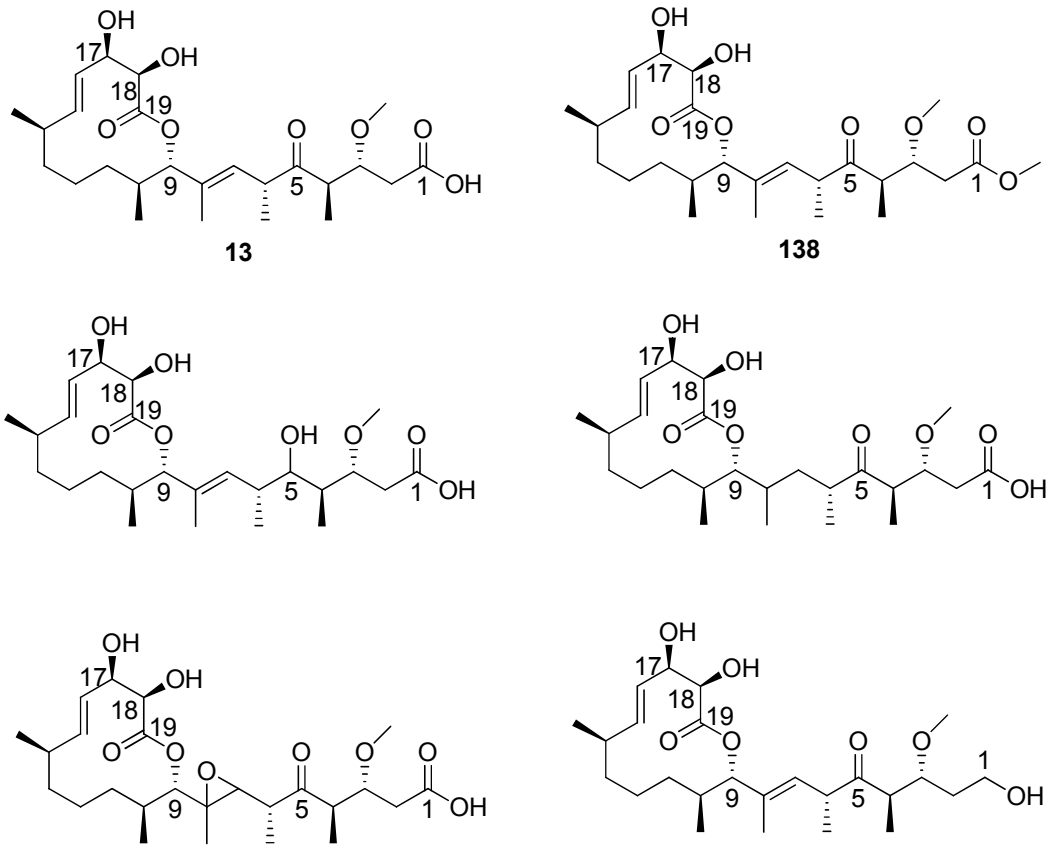


Figure 79: Derivatives of carolacton **13**

6 Experimental procedures

6.1 General Methods

6.1.1 Chemicals

Commercially available starting materials were purchased from Sigma-Aldrich Chemie GmbH (Germany), Fluka Chemie GmbH(Switzreland), Merck (Germany), Acros Organics (Belgium), TCI Organic Chemicals (Belgium), Janssen Chimica (Belgium) and Riedel de Haën AG (Switzerland) that were used without further purification except if otherwise stated. Technical solvents were distilled before use. For the reactions performed with dry solvents, THF was distilled over sodium and potassium with benzophenone, dichloromethane over calcium hydride, diethyl ether over $LiAlH_4$, ethanol and methanol over magnesium.

6.1.2 Reaction Conditions

All reactions involving water-sensitive chemicals were carried out in overnight oven dried (100 °C) clean glass equipment which was further flame dried with Bunsen burner before the start of the reaction under vacuum, then switched to nitrogen atmosphere and magnetic stirring unless otherwise stated.

6.1.3 Thin layer Chromatography and column chromatography

TLC was performed on 0.2 mm pre-coated plastic sheets of Polygram® SIL G/UV_{254} plated purchased from Macherey-Nagel. Detection of compounds was performed by immersion in 10% ethanolic solution of phosphomolybdic acid, Potassium permanganate solution or in detecting mixture containing 5 g K_2CO_3 and 5 ml 1 N NaOH in 300 ml water and followed by gentle heating with heat gun. The detection of compounds was also done under UV (254 nm) light observation.

Flash chromatography was performed on silica gel M60 (0.04-0.063 mm, 230-400 mesh ASTM) (Macherey-Nagel) under pressure with the eluent mentioned in the respective procedures.

6.1.4 Analytical techniques and devices

6.1.4.1 Gas Chromatography/Mass Spectrometry

GC-MS was performed on HP 6890 gas chromatograph coupled to an MSD 5973 (EI 70 ev) (Hewlett Packard) and on a GC 7890A coupled to an MSD 5975C (Agilent Technologies). Separation was performed in a fused-silica capillary columns BPX-5 (SGE Inc.; 25 mm x 0.22 mm I.D. x 0.25 µm) and HP5-MS (Agilent Technologies, 30 m x 0.25 mm I.D. x 0.25 µm). Instrument parameters were adjusted as follows: inlet pressure 77.1 kPa; He 23.3 ml/min; injector 250 °C; injection volume 1 µl; transfer line 300 °C; electron energy 70 eV. All of the Synthetic samples were analyzed in split mode (ratio 20:1). The maximal heating temperature for the column was 320 °C. Helium was used as carrier gas with 1.1 mL/min. The standard heating method started from 50 °C, and allowed to stay for 10 min and raised 10 °C/min until 320 °C and allowed to stay there for 5 min isothermal. The analysis was successfully carried out by using Enhanced Data Analysis D.02.00.237 program and NIST MS search 2.0 developed by the Agilent Company.

6.1.4.2 Chiral Gas Chromatography

Chiral gas chromatography was performed using a hydrodex-6-TBDMS phase (Macherey-Nagel, 25 m x 0.25 m I.D.), β-dex chiral column (length 30 m, diameter 0.32 mm, phase thickness 0.25 µm) and Lipodex-G chiral column; Length 50 m, diameter 0.25 mm, H_2 flow 1.2 mL/min.Temperature program is mentioned under the respective synthesis and analysis section of the compounds. Hydrogen was applied as carrier gas and compounds were detected with a flame ionization detector. Injector temperature 250 °C; detector temperature 300 °C; injection volume 1 µl, inlet pressure 80.0 kPa; carrier gas flow 1 mL/min; gas velocity 29 cm/sec.

6.1.4.3 Analytical conditions for LC-MS:

Coulmn: 150x2.1 mm, Hypersil Gold 3 µm C18 (Thermo); Inj. Volume: 10 µL, flowrate: 250 µL/min-, R_t = 10.9 min;

Solvents: Solvent A:[Blank], Solvent B [H2O], Solvent C [MeCN], Solvent D [2% HCOOH in MeOH/H2O 1:1],

Elution: gradient from Solvent B 92.5% solvent C 2.5% Solvent D 5% within 6.5 min to Solvent B 2.5% Solvent C 92.5% Solvent D 5% .

6.1.5 ^{1}H NMR, ^{13}C NMR experiments and optical rotations

NMR spectra were obtained with the following instruments: BRUKER DPX-200 (^{1}H 200 MHz, ^{13}C 50.5 MHz), DRX- 300 (^{1}H 300 MHz, ^{13}C 75 MHz), DRX- 400 (^{1}H 400 MHz, ^{13}C 101 MHz), or AV II-600 (^{1}H 600 MHz, ^{13}C 151 MHz). Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) as an internal standard (δ = 0 ppm). All compounds were dissolved in the respective deuterated solvents mentioned. The NMR data was analyzed using the software ACD/Labs 12.0 and TOPSPIN 3.2. Optical rotations were measured with PROPOL–Polarimeter purchased from Dr. Kernchen. The solvents used for the measurements were reported in the corresponding experimental sections.

6.1.6 Derivatization procedures

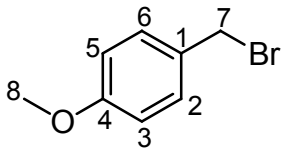
Carboxylic acids and alcohols were detected by GC-MS when derivatized with MSTFA or diazomethane. Micro scale synthesis of diazomethane in diethyl ether ((for mg amounts of analyte) was mentioned in the respective procedure and analysis section of the compounds.

Derivatization with MSTFA (N-methyl-N-(trimethylsilyl) trifluoroacetamide)

About 50 µL of MSTFA was added to the synthetic sample in dichloromethane (50 µL), followed by heating for 1 h at 60 °C. Then excess solvent and MSTFA was removed by a gentle stream of nitrogen. The residue was taken up in dichloromethane and analyzed by GC-MS.

6.2 Synthesis

6.2.1 1-(Bromomethyl)-4-methoxybenzene (36)



Phosphorous tribromide (1.6 mL, 17 mmol) was slowly added to a solution of *para*-methoxybenzyl alcohol (6.7 mL, 54 mmol) in dichloromethane (80 mL) at 0 °C and stirred for 12 h. The reaction mixture was poured into aqueous sodium bicarbonate and extracted with dichloromethane. Removal of the solvent in *vacuo* from the organic phase gave a clear oil as the product which was used in the mono protection of 1,3-propanediol (**32**).[121]

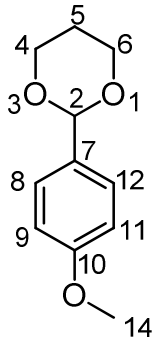
Yield: 10 g (50 mmol, 93%).

EI-MS (70 eV): *m/z* (%): 202(14), 200(15), 157(4), 122(45), 121(100), 106(19), 91(43), 90(14), 89(31), 81(8), 79(19), 78(98), 77(83), 74(7), 65(11), 63(28), 62(14), 52(41), 51(53), 50(32), 39(16).

^{1}H-NMR (200 MHz, CHLOROFORM-*d*) δ [ppm] = 7.32 (dt, *J* = 8.6, 3.0 Hz, 2 H, H-2, 6), 6.86 (dt, *J* = 8.6, 3.0 Hz, 2 H, H-3, 5), 4.50 (s, 2 H, H-7), 3.80 (s, 3 H, H-8).

^{13}C-NMR (50 MHz, CHLOROFORM-*d*) δ [ppm] = 159.7 (C-4), 130.4 (C-2, 6), 129.9 (C-1), 114.2 (C-3, 5), 55.3 (C-8), 33.9 (C-7).

6.2.2 2-(4-Methoxyphenyl)-1,3-dioxane (35)



To a solution of 1,3-propanediol **32** (8.7 g, 114 mmol) and *para*-anisaldehyde (14 mL, 115 mmol) in toluene (18 mL), a catalytic amount of PTSA (44 mg, 0.23 mmol) was added and the mixture was heated under reflux in a Dean stark apparatus for 21 h.[122] The solvent from reaction mixture was evaporated and the resulting dark brownish yellow colored crude acetal was reduced without further purification to the alcohol **37**.

Yield: 26 g (114 mmol, quant.).

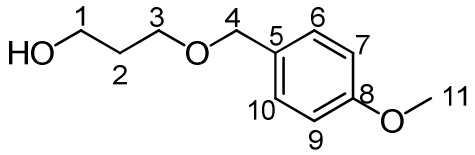
R_f = 0.38 (pentane/TBME 1:1)

EI-MS (70 eV): *m/z* (%): 194(90) $[M]^+$, 193 (98), 190 (1), 179(5), 177 (1), 165(4), 164 (17), 163 (62), 153 (4), 152 (28), 151 (2), 138 (12), 137 (57), 136 (94), 135 (100), 133(5), 122(4), 121 (38), 119 (8), 115 (1), 110 (4), 108 (56), 107 (45), 105 (28), 94 (26), 92(57), 91(15), 87 (33), 79(17), 77(71), 73 (1), 65 (40), 63 (29), 59(13), 53(7), 51(26), 42(23), 39 (23).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.37 - 7.43 (m, 2 H, H-8, 12), 6.85 - 6.91 (m, 2 H, H-9, 11), 5.45 (s, 1 H, H-2), 4.21 - 4.28 (m, 2 H, H-4), 3.92 - 4.01 (m, 2 H, H-6), 3.79 (s, 3 H, H-14), 2.12 - 2.29 (m, 1 H, H-5), 1.42 (dtt, *J*=13.5, 2.6, 1.4 Hz, 1 H, H-5).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 159.9 (C-10), 131.3 (C-7), 127.2 (C-8, 12), 113.5 (C-9, 11), 101.5 (C-2), 67.3 (C-4, 6), 55.2 (C-14), 25.7 (C-5).

6.2.3 3-((4-Methoxybenzyl)oxy)propan-1-ol (37)



To a suspension of NaH (2.3 g, 57 mmol, 60 % in mineral oil, washed with dry hexane before use) in dry DMSO (30 mL) stirred at 0 °C under an inert atmosphere, 1,3-propanediol (**32**) (51 g, 67 mmol) was added dropwise while stirring. The resulting mixture was stirred for 15 min and to this solution *para*-methoxybenzyl bromide (**36**) (7 mL, 48 mmol) was added dropwise and allowed to stir at room temperature for 18 h 30 min and monitored by TLC. The reaction mixture was quenched by adding 50 mL of water, extracted with TBME, and the organic phase was washed with brine, dried with $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography with 2:1 pentane/TBME mixture as eluent to furnish the product **37** as colorless oil.[123]

Yield: 5 g (25 mmol, 53%).

Reduction of acetal **35** *to alcohol* **37:**

To a solution of crude acetal **13** (3.3 g, 17 mmol) in toluene (6 mL) cooled in an ice/salt bath, DIBAL-H (19 mL, 1M in toluene) was added dropwise at such a rate as to maintain the reaction mixture temperature at 0 °C. The reaction mixture was stirred at room temperature overnight, diluted with toluene (5 mL), and cooled in an ice/water bath. Methanol (3 mL) was added dropwise at such a rate as to keep the temperature below 40 °C.[56] Water (3 mL) was then added dropwise and the reaction mixture stirred at room temperature for 1 h. The white precipitate was filtered through a pad of celite and thoroughly washed with toluene. The filtrate was concentrated to give the crude yellow oil, purified by flash chromatography with 2:1 pentane/TBME mixture as eluent to furnish the alcohol **37** as colorless oil.

Yield: 3 g (15 mmol, 90%).

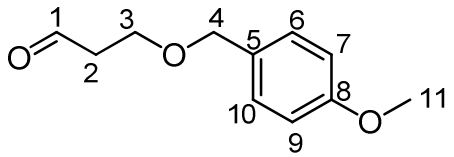
R_f = 0.12 (pentane/TBME 1:1).

EI-MS (70 eV): *m/z* (%): 196(7) $[M]^+$, 195(1), 177(1), 137(85), 135(6), 121(100), 109(11), 107(7), 91(7), 89(59), 77(17), 65(4), 52(3), 41(2), 39(2).

^{1}H-NMR (200 MHz, CHLOROFORM-*d*) δ [ppm] = 7.25 (dt, *J* = 8.6, 2.8 Hz, 2 H, H-6, 10), 6.88 (dt, *J* = 8.8, 2.8 Hz, 2 H, H-7, 9), 4.45 (s, 2 H, H-4), 3.80 (s, 3 H, H-11), 3.75 (t, *J* = 5.6 Hz, 2 H, H-3), 3.63 (t, *J* = 5.8 Hz, 2 H, H-1), 2.43 (t, *J* = 4.8 Hz, 1 H, -OH), 1.84 (quin, *J* = 5.7 Hz, 2 H, H-2).

^{13}C-NMR (50 MHz, CHLOROFORM-*d*) δ [ppm] = 159.2 (C-8), 130.2 (C-5), 129.2 (C-6, 10), 113.8 (C-7, 9), 72.9 (C-4), 69.0 (C-3), 61.8 (C-1), 55.2 (C-11), 32.1 (C-2).

6.2.4 3-((4-Methoxybenzyl)oxy)propanal (41)



The alcohol **37** (5 g, 26 mmol) was dissolved in dry DMSO (52 mL) and cooled to 0 °C prior to the addition of IBX (15 g, 52 mmol) portionwise while stirring under an inert atmosphere.

The reaction mixture was allowed to stir at RT and monitored with TLC until the starting material is consumed. The reaction is quenched by addition of water (70 mL) and the formed precipitate was filtered through a Buchner funnel using water jet pump. The aqueous extract was extracted thrice with diethyl ether. The combined organic extracts were washed with brine and dried with Na_2SO_4, filtered, and concentrated in *vacuo* to get the crude product as yellow colored oil. The crude product was purified by flash chromatography with 5:1 pentane/TBME mixture as eluent to get the desired aldehyde **41** as light yellow colored oil.

Yield: 4 g (21 mmol, 80%).

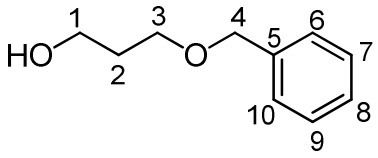
R_f = 0.22 (pentane/TBME 1:1).

EI-MS (70 eV): *m/z* (%): 194(13) $[M]^+$, 176(1), 163(1), 149(1), 138(13),137(72), 135(7), 122(11), 121(100), 109(16), 107(6), 94(9), 89(5), 78(17), 77(22), 65(5), 63(4), 55(2), 52(4), 51(6), 41(2), 39(4).

^{1}H-NMR (200 MHz, CHLOROFORM-*d*) δ [ppm] = 9.78 (t, *J* = 1.9 Hz, 1 H, H-1), 7.25 (dt, *J* = 8.8, 2.8 Hz, 2 H, H-6, 10), 6.88 (dt, *J* = 8.8, 2.8 Hz, 2 H, H-7, 9), 4.46 (s, 2 H, H-4), 3.80 (s, 3 H, H-11), 3.78 (t, *J* = 6.3 Hz, 2 H, H-3), 2.67 (td, *J* = 6.1, 1.8 Hz, 2 H, H-2).

^{13}C-NMR (50 MHz, CHLOROFORM-*d*) δ [ppm] = 201.1 (C-1), 159.3 (C-8), 129.9 (C-5), 129.3 (C-6, 10), 113.8 (C-7, 9), 72.9 (C-4), 63.5 (C-3), 55.2 (C-11), 43.8 (C-2).

6.2.5 3-(Benzyloxy)propan-1-ol (38)



To a solution of NaH (590 mg, 14.7 mmol, 60 % in mineral oil, washed with dry hexane before use) in dry DMF (78 mL) cooled at 0 °C under inert atmosphere, 1,3-propanediol (**32**) (1 g, 13.4 mmol) was added dropwise while stirring and stirred for another 10 min. Benzylbromide (1.6 mL, 13.4 mmol) was added dropwise cautiously and the mixture was allowed to warm up to room temperature and stirred for 16 h.[49] The reaction was quenched by addition of water (25 mL) and subsequently extracted four times with ethyl acetate. The organic layer was dried with $MgSO_4$, filtered, and concentrated under reduced pressure to

afford a yellow oil. Flash chromatography with 5:1 hexane/ethyl acetate mixture resulted in the 3-benzyloxypropan-1-ol (**38**) as pale yellow oil.

Yield: 2 g (12 mmol, 90%).

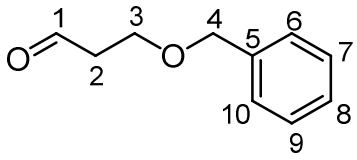
R_f = 0.38 (hexane/ethyl acetate 1:1).

EI-MS (70 eV): *m/z* (%): 166(2) $[M]^+$, 147(3), 130(1), 120(2), 117(2), 108(8), 107(74), 105(9), 92(17), 91(100), 89(8), 80(2), 79(23), 77(19), 65(24), 63(7), 57(4), 51(10), 50(6), 43(9), 41(5), 39(12), 38(3).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.36-7.25 (m, 5 H, H(6-10)), 4.51 (s, 2 H, H-4), 4.20 (br, s, 1 H, *OH*), 3.77 (t, *J* = 5.8 Hz, 2 H, H-3), 3.64 (td, *J* = 6.2, 5.2 Hz, 2 H, H-1), 1.85 (quint, *J* = 6.2 Hz, 2 H, H-2).

^{13}C-NMR (100 MHz, CHLOROFORM-*d*) δ [ppm] = 137.9 (C-5), 128.4 (C-7, 9), 127.6 (C-6, 8, 10), 73.2 (C-4), 69.0 (C-3), 61.5(C-1), 31.9 (C-2).

6.2.6 3-(Benzyloxy)propanal (42)



To a solution of alcohol **38** (2 g, 12 mmol) in dichloromethane (11 mL) and DMSO (9 mL) at 0 °C under an inert atmosphere and stirring, triethylamine (14.5 mL, 103 mmol) was added dropwise slowly. In a separate flask DMSO (19 mL) was added to pyridine- sulfurtrioxide complex (Py•SO_3) (5 g, 31.2 mmol), stirred under an inert atmosphere for 15min, transferred to the flask containing alcohol **38** slowly and stirred again at 0 °C for 5 h. The reaction mixture was monitored by TLC, quenched by addition of water (30 mL) and diluted with ethyl acetate. The organic phase was extracted 4 times with ethyl acetate, washed with NH_4Cl solution, brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford aldehyde **42** as a crude product. Flash chromatography of the crude product with 5:1 hexane/ethyl acetate mixture afforded aldehyde **20** as colorless oil.

Yield: 1.6 g (10 mmol, 80%).

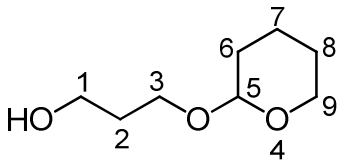
R_f = 0.38 (hexane/ethyl acetate 3:1).

EI-MS (70 eV): *m/z* (%): 164(1)[M]$^+$, 146(1), 120(7), 108(12), 107(90), 92(15), 91(100), 89(7), 79(38), 77(25), 73(4), 65(21), 63(7), 57(8), 51(16), 50(7), 45(4), 39(14), 38(3).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 9.78 (t, *J* = 1.8 Hz, 1 H, H-1), 7.37-7.25 (m, 5 H, H(6-10)), 4.52 (s, 2 H, H-4), 3.8 (t, *J* = 6 Hz, 2 H, H-3), 2.68 (td, *J* = 6.1, 1.9 Hz, 2 H, H-2).

^{13}C-NMR (100 MHz, CHLOROFORM-*d*) δ [ppm] = 201.1 (C-1), 137.8 (C-5), 128.4 (C-7, 9), 127.6 (C-6, 8, 10), 73.2 (C-4), 63.8 (C-3), 43.8 (C-2).

6.2.7 3-((Tetrahydro-2H-pyran-2-yl)oxy)propan-1-ol (40)



A solution of 1,3-propanediol (**32**) (4 g, 54 mmol) and 3,4-dihydro-2*H*-pyran (0.91 g, 10.82 mmol) in dry dichloromethane (22 mL) was stirred with a catalytic amount of pyridinium-*para*-toluene sulfonate (PPTS) (33 mg, 0.13 mmol) at room temperature for 4 h. The reaction mixture was quenched by addition of water (30 mL), and the aqueous phase was extracted (3 x 20 mL) with CH_2Cl_2. The organic phases were washed with brine, dried with $MgSO_4$, filtered, and concentrated in *vacuo* to afford the crude alcohol.[124] Purification of the crude product by flash chromatography with 1:1 pentane/diethyl ether mixture as eluent provided alcohol **40** as colorless oil.

Yield: 0.9 g (5.6 mmol, 52%).

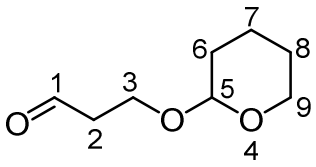
R_f = 0.20 (pentane/diethyl ether 1:1).

EI-MS (70 eV): *m/z* (%): 160(1) [M]$^+$, 159 (4), 115(1), 105(3), 102(5), 101(39), 87(26), 85(100), 84(16), 83(6), 77(2), 75(1), 74(5), 73(1), 69(2), 67(16), 65(1), 59(27), 58(18), 57(39), 56(31), 55(31), 53(6), 51(3), 47(4), 45(7), 44(11), 43(27), 42(12), 41(62), 40(6).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 4.57 - 4.62 (m, 1 H, H-5), 3.82 - 3.98 (m, 2 H, H-9), 3.79 (td, *J*=6.1, 0.4 Hz, 2 H, H-1), 3.48 - 3.64 (m, 2 H, H-3), 2.51 (br. s., 1 H, *OH*), 1.81 - 1.91 (m, 2 H, H-2), 1.66 - 1.80 (m, 2 H, H-6), 1.47 - 1.63 (m, 4 H, H-7, 8).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 99.1 (C-5), 66.2 (C-3), 62.5 (C-9), 61.4 (C-1), 32.0 (C-2), 30.6 (C-6), 25.3 (C-8), 19.6 (C-7).

6.2.8 3-((Tetrahydro-2H-pyran-2-yl)oxy)propanal (44)



Oxalylchloride (0.809 g, 6.4 mmol) was dissolved in dichloromethane (29 mL), cooled to -78 °C, and stirred under an inert atmosphere. DMSO (1.04 g, 13.3 mmol) dissolved in dichloromethane (2 mL) was added slowly dropwise *via* syringe pump over a period of 10 minutes to the reaction mixture and stirred for 30 minutes. The alcohol **40** (0.85 g, 5.3 mmol) dissolved in dichloromethane (9 mL) was added slowly via a syringe pump over a period of 25 minutes. After stirring the solution for 1 h at -78 °C, triethylamine (2.15 g, 21.3 mmol) was added. The resulting thick white mixture was warmed to room temperature over a period of 1 h followed by the addition of water (20 mL). The aqueous layer was extracted (3 x 20 mL) with dichloromethane. The separated organic phases were washed with water, saturated sodium chloride, dried with $MgSO_4$ and filtered. After removal of the solvent under *vacuo*, the crude product was purified by flash chromatography on silica gel with 2:1 pentane/diethyl ether mixture as eluent to afford the desired aldehyde **44** as colorless oil.

Yield: 0.67 g (4.25 mmol, 80%).

R_f = 0.56 (pentane/diethyl ether 2:1).

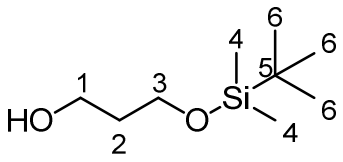
EI-MS (70 eV): *m/z* (%): 158(1) $[M]^+$, 157(3), 140(1), 127(1), 113(1), 102(5), 101(53), 86(6), 85(100), 84(16), 83(9), 81(1), 75(2), 74(16), 73(3), 72(15), 69(3), 67(20), 65(1), 57(80), 56(54), 55(54), 53(9), 51(4), 45(10), 44(25), 43(55), 41(98), 39(42), 38(5).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 9.82 (t, *J*=1.9 Hz, 1 H, H-1), 4.63 (t, *J*=3.5 Hz, 1 H, H-5), 4.10 (dt, *J*=10.2, 6.1 Hz, 1 H, H-3), 3.81 - 3.88 (m, 1H, H-9), 3.76 (dt, *J*=10.4,

6.0 Hz, 1 H, H-3), 3.49 - 3.56 (m, 1H, H-9), 2.70 (td, *J*=6.1, 1.9 Hz, 2 H, H-2), 1.62 – 1.83 (m, 2H, H-6), 1.49 - 1.61 (m, 4H, H-7, 8).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 201.3 (C-1), 98.9 (C-5), 62.2 (C-9), 61.2 (C-3), 43.8 (C-2), 30.4 (C-6), 25.3 (C-8), 19.3 (C-7).

6.2.9 3-((*tert*-Butyldimethylsilyl)oxy)propan-1-ol (39)



To a suspension of NaH (344 mg, 8.6 mmol, 60 % in mineral oil, washed with dry hexane before use) in THF (20 mL) stirred under inert atmosphere, 1,3-propanediol (**32**) (654 mg, 8.6 mmol) was added dropwise and stirred at room temperature for 45 min followed by the addition of TBDMSCl (1.3 g, 8.6 mmol) and stirred further 45 min at room temperature.[50,51] The resultant mixture was extracted (3 x 20 mL) with TBME, the organic phases were washed with 10 % aq. K_2CO_3, brine, dried with $MgSO_4$, filtered, and concentrated in *vacuo*. The crude product was purified by flash chromatography with 4:1 pentane/ethyl acetate mixture as eluent to afford the alcohol **39** as colorless oil.

Yield: 1.60 g (8.42 mmol, 98%).

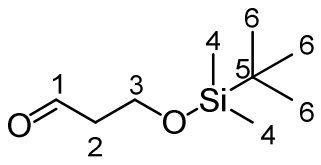
R_f = 0.41 (pentane/ethyl acetate 4:1).

EI-MS (70 eV): *m/z* (%): 189((1) $[M-1]^+$, 175(1), 147(2), 133(53), 117(7), 115(9), 105(91), 101(7), 91(9), 87(13), 85(4), 76(21), 75(100), 73(24), 72(4), 61(9), 59(17), 57(12), 55(3), 53(1).

^{1}H-NMR (200 MHz, CHLOROFORM-*d*) δ [ppm] = 3.70 - 3.79 (m, 4 H, H-1, 3), 2.58 (br. s., 1 H, *OH*), 1.70 (quin, *J*=5.6 Hz, 2 H, H-2), 0.82 (s, 9 H, H-6), 0.00 (s, 6H, H-4).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 62.8 (C-1), 62.3 (C-3), 34.2 (C-2), 25.8 (C-6), 18.2 (C-5), -5.5 (C-4).

6.2.10 3-((*tert*-Butyldimethylsilyl)oxy)propanal (43)



Oxalylchloride (802 mg, 6.3 mmol) was dissolved in dichloromethane (29 mL) and cooled to -78 °C stirring under inert atmosphere. DMSO (1.03 g, 13.2 mmol) dissolved in dichloromethane (2 mL) was added slowly dropwise *via* syringe pump over a period of 10 minutes to the reaction mixture and stirred for 30 minutes followed by the addition of alcohol **39** (1 g, 5.3 mmol) dissolved in dichloromethane (8 mL) and slowly added via a syringe pump over a period of 25 minutes. After stirring the solution for 1 h at -78 °C, triethylamine (2.13 g, 21.1 mmol) was added. The resulting thick white mixture was warmed to room temperature over a period of 1 h followed by the addition of water (20 mL). The aqueous layer was extracted (3 x 20 mL) with dichloromethane. The separated organic phases were washed with water, saturated sodium chloride, dried with $MgSO_4$ and filtered. After removal of the solvent under *vacuo*, the crude product was purified by flash chromatography on silica gel with 9:1 pentane/diethyl ether mixture as eluent to afford the desired aldehyde **43** as colorless liquid.

Yield: 890 mg (4.73 mmol, 90%).

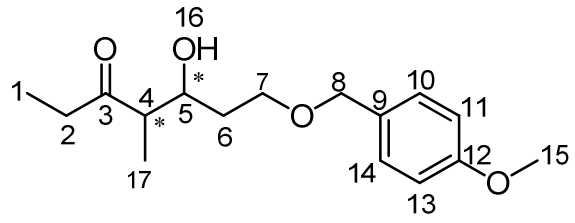
R_f = 0.43 (pentane/diethyl ether 9:1).

EI-MS (70 eV): *m/z* (%): 187(1) $[M-1]^+$, 143(1), 133(9), 132(24), 131(100), 129(4), 117(18), 115(9), 103(11),102(22), 101(100), 99(6), 89(6), 87(8), 85(5), 77(5), 76(7), 75(86), 73(20), 71(6), 61(9), 60(7), 59(53), 58(8), 57(14), 55(5), 47(12), 45(22), 43(9), 42(4), 41(22).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 9.73 (t, *J*=2.1 Hz, 2 H, H-1), 3.92 (t, *J*=6.1 Hz, 2 H, H-3), 2.53 (td, *J*=6.0, 2.1 Hz, 2 H, H-2), 0.81 (s, 9 H, H-6), 0.00 (s, 6 H, H-4).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 202.0 (C-1), 57.4 (C-3), 46.5 (C-2), 25.8 (C-6), 18.2 (C-5), -5.5 (C-4).

6.2.11 5-Hydroxy-7-((4-methoxybenzyl)oxy)-4-methylheptan-3-one (45 a)



Preparation of LDA solution:

n-BuLi (1 mL, 1.7 mmol, 1.6 M in hexane) was slowly added dropwise to the solution of diisopropylamine (0.3 mL, 2.3 mmol) in dry THF (3 mL) at -78 °C and stirred for 30 minutes.

To the freshly prepared LDA solution under inert atmosphere, diethylketone **31** (121 mg, 1.4 mmol) in THF (2 mL) was added dropwise at -78 °C and stirred for 3 h followed by the addition of aldheyde **41** (398 mg, 2.05 mmol) in dry THF(5 mL) at the same temperature. The mixture was stirred for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate (3 X 30 mL). The organic phases were washed with brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography with 5:1 pentane/ethyl acetate mixture as eluent to afford the aldol product (mixture of diastereomers) **45a** as colorless oil.

Yield: 290 mg (1 mmol, 75%, d.r. = 4:1).

R_f = 0.21 (pentane/ethyl acetate 2:1).

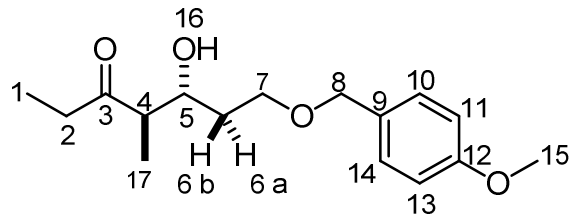
EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 352(1) $[M]^+$, 334(1), 267(5), 262(3), 231(2), 217(2), 215(8), 210(7), 209(32), 187(16), 177(20), 176(72), 175(7), 170(5), 159(5), 158(13), 157(17), 144(9), 143(11), 141(18), 138(9), 137(55), 136(19), 135(28), 132(5), 131(30), 130(19), 129(12), 127(8), 126(46), 125(11), 123(7), 122(52), 121(100), 117(8), 115(22), 111(8), 109(11), 108(14), 107(9), 106(7), 103(14), 101(11), 97(17), 94(6), 92(6), 91(21), 90(9), 89(10), 85(5), 79(8), 78(24), 77(32), 76(6), 75(48), 74(7), 73(48), 69(20), 67(6), 65(7), 61(6), 59(10), 57(61), 44(10), 41(8).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.22 - 7.26 (2H, m, H-10, 14), 6.85 - 6.89 (2H, m, H-11, 13), 4.44 (2H, s, H-8), 4.07 (1H, ddd, $J_{5,6}$ = 9.8 Hz, $J_{5,4}$ = 7.3 Hz, J = 3.3 Hz, H-5, major *ds*), 3.90 (1H, dt, $J_{5,6}$ = 7.8 Hz, $J_{5,16}$ = 3.8 Hz, H-5, minor *ds*), 3.80 (3H, s, H-15), 3.56 - 3.70 (2H, m, H-7), 3.38 (1H, d, $J_{16,5}$ = 3.8 Hz, H-16), 2.62 (1H, dq, $^3J_{4,5}$ = 7.3 Hz, $^3J_{4,17}$

= 7.0 Hz, H-4), 2.51 (2H, dq, ^{2}J = 17.8 Hz, $^{3}J_{2,1}$ = 7.3 Hz, H-2), 1.47 - 1.95 (2H, m, H-6), 1.11 (3H, d, $^{3}J_{17,4}$ = 7.0 Hz, H-17), 1.03 (3H, t, $^{3}J_{1,2}$= 7.3 Hz, H-1).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 215.6 (C-3), 159.2 (C-12), 130.0 (C-9), 129.3 (C-10, 14), 113.8 (C-11, 13), 72.9 (C-8), 70.9 (C-5), 68.4 (C-7), 55.2 (C-15), 50.5 (C-4), 35.4 (C-2), 33.7 (C-6), 11.1 (C-17), 7.5 (C-1).

6.2.12 (4*RS*,5*RS*)-5-Hydroxy-7-((4-methoxybenzyl)oxy)-4-methylheptan-3-one (45)



To a solution of dicyclohexylboron chloride (18 mL, 18 mmol, 1M in hexane) in dry diethyl ether (20 mL) stirred at 0 °C under inert atmosphere triethylamine (2.44 g, 24.10 mmol) was added dropwise. After stirring for 1 h diethylketone **31** (1.38 g, 16.05 mmol) in dry diethyl ether (18 mL) was added dropwise and stirred at 0 °C for 4 h. The reaction mixture was cooled to -78 °C, aldehyde **41** (4.4 g, 22.47 mmol) in dry diethyl ether (30 mL) was added dropwise over a period of 30 min and the mixture was stirred for 2 h. The reaction mixture was left overnight (16 h) in a freezer at -32 °C. The reaction mixture was quenched at 0 °C by addition of pH 7 buffer (30 mL) and extracted with diethyl ether (3 x 20 mL). The combined organic layers were concentrated in *vacuo*. The crude oil was suspended in methanol (25 mL) and pH 7 buffer (5 mL) followed by dropwise addition of hydrogen peroxide (1.5 mL, 30% aq.) under cooling at 0 °C. The reaction mixture was stirred for 2 h, then poured into water (20 mL) and extracted with dichloromethane (3 x 20 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, brine, dried with $MgSO_4$, filtered and concentrated in *vacuo*.[63] The crude product was concentrated by flash column chromatography (10:1 pentane/ethyl acetate mixture) to afford aldol product **45** as colorless oil. The C4/C5 *anti*-arrangement can be deduced by the coupling constant $^{3}J_{4,5}$ = 9.1 Hz.

Yield: 4 g (14.3 mmol, 89%, 96% *d.e.*).

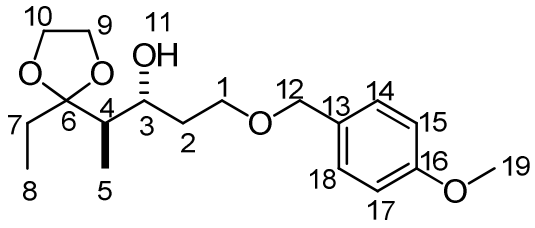
R_f = 0.21 (pentane/ethyl acetate 2:1).

EI-MS (70 eV): *m/z* (%): 262(1) $[M-18]^+$, 228(1), 217(1), 205(1), 197(1), 176(1), 161(1), 138(2), 137(3), 136(5), 135(7), 127(3), 126(30), 124(3), 123(2), 122(16), 121(100), 116(1), 113(3), 111(14), 108(3), 106(4), 97(15), 95(5), 91(6), 89(2), 83(2), 79(4), 78(7), 77(10), 67(6), 65(4), 63(2), 57(4), 55(4), 43(2), 41(4), 39(4).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.13 - 7.17 (2H, m, H-14, 10), 6.76 - 6.81 (2H, m, H-13, 11), 4.35 (2H, s, H-8), 3.82 (1H, dt, $^3J_{5,6a}$ = 12.4, $^3J_{5,4}$ = 9.1 Hz, *J* = 7.1 Hz, H-5), 3.71 (3H, s, H-15), 3.57 - 3.63 (1H, m, H-7), 3.50 - 3.57 (1H, m, H-7), 3.33 (1H, d, $^3J_{16,5}$ = 4.3 Hz, H-16, anti *ds*), 3.28 (1H, d, $^3J_{16,5}$ = 2.5 Hz, H-16 syn *ds*), 2.57 (1H, dq, $^3J_{4,5}$ = 9.1 Hz, $^3J_{4,17}$ = 7.1 Hz, H-4), 2.37 - 2.51 (2H, m, H-2), 1.67 - 1.76 (1H, m, H-6 b), 1.54 - 1.65 (1H, m, H-6 a), 0.98 (3H, d, $^3J_{17,4}$ = 7.1 Hz, H-17), 0.95 (3H, t, $^3J_{1,2}$ = 7.3 Hz, H-1).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 215.6 (C-3), 159.2 (C-12), 129.9 (C-9), 129.2 (C-14, 10), 113.7 (C-11, 13), 73.04 (C-8), 73.02 (C-5), 68.2 (C-7), 55.1 (C-15), 51.1 (C-4), 35.9 (C-2), 33.8 (C-6), 13.5 (C-17), 7.3 (C-1).

6.2.13 (3*RS*,4*RS*)-4-(2-Ethyl-1,3-dioxolan-2-yl)-1-((4-methoxybenzyl)oxy)pentan-3-ol (48)



Aldol product **45** (100 mg, 0.36 mmol) was dissolved in ethylene glycol (2 mL) and stirred under inert atmosphere at room temperature. To the above solution triethylorthoformate (267 mg, 1.8 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (1.5 mg) was added and stirred at room temperature for 2 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with chloroform (3 x 20 mL), dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography with pentane/ethyl acetate mixture (gradient elution 10:1 to 2:1) to afford the **48** as colorless oil with fruity odour.

Yield: 100 mg (0.31mmol, 86%).

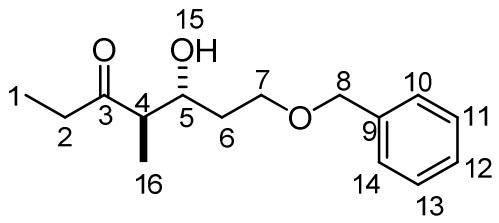
R_f = 0.23 (pentane/ethyl acetate 2:1).

EI-MS (70 eV): *m/z* (%): 324(2) $[M]^+$, 306(3), 295(2), 279(5), 278(6), 277(27), 261(15), 250(16), 244(4), 221(6), 206(9), 203(15), 194(26), 189(12), 185(11), 176(24), 170(11), 164(12), 159(48), 143(82), 141(71), 137(83), 135(48), 130(27), 126(57), 125(26), 122(72), 121(98), 119(16), 115(19), 113(13), 109(34), 108(19), 107(25), 102(55), 101(100), 100(46), 99(35), 97(20), 94(23), 91(41), 89(24), 87(63), 85(23), 79(17), 78(54), 77(60), 73(24), 69(27), 65(17), 57(85), 55(21), 45(24), 43(17), 41(25), 39(10).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.10 - 7.23 (2H, m, H-18, 14), 6.71 - 6.87 (2H, m, H-17, 15), 4.28 - 4.44 (2H, m, H-12), 4.09 - 4.25 (1H, m, H-3), 3.82 - 3.96 (4H, m, H-10, 9), 3.72 (3H, s, H-19), 3.57 (2H, t, *J* = 6.6 Hz, H-1), 3.18 (1H, br. s., H-11), 1.82 - 1.91 (1H, m, H-4), 1.72 - 1.82 (2H, m, H-2), 1.59 - 1.69 (2H, m, H-7), 1.18 (3H, t, *J* = 7.2 Hz, H-8), 0.83 (3H, d, *J* = 7.2 Hz, H-5).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 159.0 (C-16), 130.5 (C-13), 129.2 (C-14, 18), 114.5 (C-6), 113.7 (C-15, 17), 72.7 (C-12), 70.3 (C-1), 67.4 (C-3), 65.3 (C-10, 9), 55.2 (C-19), 44.2 (C-4), 34.4 (C-2), 25.7 (C-7), 12.0 (C-5), 6.8 (C-8).

6.2.14 (4*RS*,5*RS*)-7-(Benzyloxy)-5-hydroxy-4-methylheptan-3-one (46)



To a solution of dicyclohexylboron chloride (0.5 mL, 0.5 mmol, 1M in hexane) in dry diethyl ether (1 mL) stirred at 0 °C under inert atmosphere triethylamine (64 mg, 0.63 mmol) was added dropwise. After stirring for 1 h the addition of diethylketone **31** (36 mg, 0.42 mmol) in dry diethyl ether (1 mL) followed and stirring continued at 0 °C for 4 h. The reaction mixture was cooled down to -78 °C, aldehyde **42** (114 mg, 0.6 mmol) in dry diethylether (1 mL) was added dropwise and the mixture stirred for 2 h. The reaction mixture was left overnight (16 h) in a freezer at approximate temperature -32 °C. The reaction mixture was quenched at 0 °C by addition of pH 7 buffer (2 mL) and extracted with diethylether (3 x 10 mL). The combined organic layers were concentrated in *vacuo*. The crude oil was suspended in methanol (5 mL) and pH 7 buffer (1 mL) followed by addition of hydrogen peroxide (1.5 mL, 30% aq.), added dropwise at 0 °C. The reaction mixture was stirred for 2 h, then poured into water (10 mL) and extracted with dichloromethane (3 x 10 mL). The combined organic layers were washed

with saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, filtered and concentrated in *vacuo*. The crude product was concentrated by flash column chromatography (pentane/ethyl acetae 10:1) to afford aldol product **46** as colorless oil.

Yield: 86 mg (0.34 mmol, 82%, 98% *d.e.*).

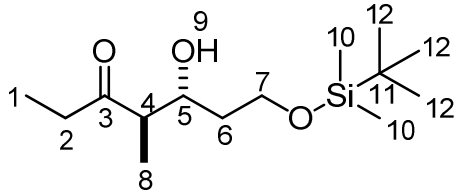
R_f = 0.2 (pentane/ethyl acetate 2:1).

EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 322(2) $[M]^+$, 307(1) $[M-15]^+$, 293(1), 277(1), 263(1), 249(1), 237(1), 232(1), 215(1), 201(1), 187(2), 173(4), 171(3), 158(3), 143(4), 131(8), 126(5), 115(4), 107(8), 105(4), 101(3), 92(10), 91(100), 89(4), 65(17), 63(3), 59(4), 57(24), 51(5), 45(8), 41(6), 39(7).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.28 - 7.38 (5H, m, H-10, 11, 12, 13, 14), 4.51 (2H, s, H-8), 3.94 (1H, ddd, *J* = 7.8 Hz, 7.0 Hz, 4.0 Hz, H-5), 3.59 - 3.68 (2H, m, H-7), 3.45 (1H, br. s., H-15), 2.67 (1H, quin, *J* = 7.1 Hz, H-4), 2.45 - 2.58 (2H, m, H-2), 1.65 - 1.77 (2H, m, H-6), 1.08 (3H, d, *J* = 7.2 Hz, H-16), 1.04 (3H, t, *J* = 7.3 Hz, H-1).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 216.1 (C-3), 137.8 (C-9), 128.4 (C-11, 13), 127.7 (C-12), 127.7 (C-14, 10), 73.3 (C-8), 70.4 (C-5), 68.6 (C-7), 51.1 (C-4), 36.0 (C-2), 33.8 (C-6), 13.6 (C-16), 7.4 (C-1).

6.2.15 (4*RS*,5*RS*)-7-((*tert*-Butyldimethylsilyl)oxy)-5-hydroxy-4-methylheptan-3-one (47)



To a solution of dicyclohexylboron chloride (0.2 mL, 0.2 mmol, 1M in hexane) in dry diethylether (0.5 mL) stirred at 0 °C under inert atmosphere triethylamine (28 mg, 0.27 mmol) was added dropwise. After stirring for 1 h the addition of diethylketone **31** (16 mg, 0.18 mmol) in dry diethyl ether (0.5 mL) followed and stirring continued at 0 °C for 4 h. The reaction mixture was cooled down to -78 °C, aldehyde **43** (59 mg, 0.25 mmol) in dry diethylether (0.4 mL) was added dropwise, and the mixture stirred for 2 h. The reaction mixture was left overnight (16 h) in a freezer at approximate temperature -32 °C. The reaction mixture was quenched at 0 °C by addition of pH 7 buffer (1 mL) and extracted with

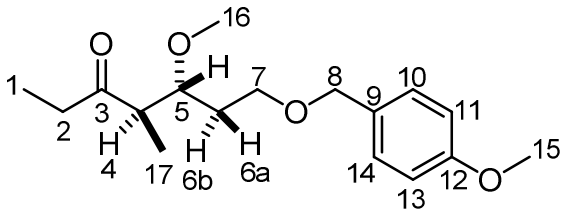
diethylether (3 x 5 mL). The combined organic layers were concentrated in *vacuo*. The crude oil was suspended in methanol (2 mL) and pH 7 buffer (0.4 mL) followed by addition of hydrogen peroxide (0.6 mL, 30% aq.), added dropwise at 0 °C. The reaction mixture was stirred for 2 h, then poured into water (6 mL) and extracted with dichloromethane (3 x 6 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, filtered, and concentrated in *vacuo* to afford crude aldol product **47**.

Yield: 40 mg (0.15 mmol, 80%, 81% *d.e.*).

R_f = 0.15 (pentane/ethyl acetate 2:1).

EI-MS (70 eV): *m/z* (%): 273(1) $[M-1]^+$, 245(1), 229(1), 211(1), 201(2), 193(1), 189(1), 176(1), 173(2), 171(2), 144(2), 143(8), 137(15), 135(3), 122(10), 121(100), 115(5), 109(4), 107(2), 101(2), 99(2), 94(2), 91(6), 89(3), 78(10), 7(12), 75(27), 73(8), 57(20), 41(10), 39(4).

6.2.16 (4*RS*,5*RS*)-5-Methoxy-7-((4-methoxybenzyl)oxy)-4-methylheptan-3-one (49)



To a solution of proton sponge® (7.3 g, 34 mmol) and trimethyloxonium tetrafluoroborate (5 g, 34 mmol) in dichloromethane (130 mL), a solution of aldol **45** (1.2 g, 4.25 mmol) in dichloromethane (8 mL) was added at room temperature. The reaction mixture was stirred for 18 h and saturated aqueous $NaHCO_3$ was added. Diethyl ether was added until the organic layer stayed on top. Layers were separated and the aqueous layer was extracted with diethylether (3 x 100 mL).The combined organic layers were washed several times with an aqueous $KHSO_4$ solution (1.5 M) until the color of the organic layer faded from brown to nearly colorless. Then the organic layer was washed with brine, dried with $MgSO_4$ and concentrated in *vacuo*.[68] Purification by flash column chromatography with pentane/ethyl acetate 10:1 provided the methylated product **49** as colorless oil.

Yield: 1 g (3.4 mmol, 80%).

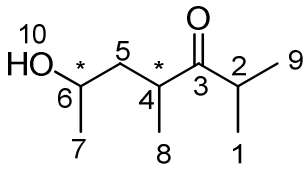
R_f = 0.68 (pentane/TBME 1:1).

EI-MS (70 eV): *m/z* (%): 294(1) $[M]^+$, 262(2), 205(2), 176(11), 141(2), 138(2), 137(12), 136(5), 135(11), 126(4), 124(5), 122(11), 121(100), 109(5), 107(4), 97(4), 92(4), 91(12), 89(5), 86(2), 81(4), 79(13), 78(16), 77(22), 69(5), 67(11), 65(10), 63(9), 57(26), 53(10), 51(11), 41(14), 39(22).

^{1}H-NMR (600 MHz, CHLOROFORM-*d*) δ [ppm] = 7.24 - 7.28 (2H, m, H-14, 10), 6.86 - 6.90 (2H, m, H-13, 11), 4.43 (2H, s, H-8), 3.81 (3H, s, H-15), 3.58 (1H, dt, *J* = 8.1 Hz, 3.6 Hz, H-5), 3.50 - 3.56 (2H, m, H-7), 3.27 (2H, s, H-16), 2.78 (1H, qd, *J* = 7.2 Hz, 2.6 Hz, H-4), 2.49 (2H, dq, *J* = 7.3 Hz, 2.8 Hz, H-2), 1.84 (1H, dtd, *J* = 14.5 Hz, 7.2 Hz, 3.2 Hz, H-6a), 1.65 (1H, ddt, *J* = 13.9 Hz, 7.9 Hz, 6.0 Hz, H-6b), 1.04 (3H, t, *J* = 7.2 Hz, H-1), 1.00 (3H, d, *J* = 7.0 Hz, H-17).

^{13}C-NMR (150 MHz, CHLOROFORM-*d*) δ [ppm] = 214.1 (C-3), 159.2(C-12), 130.5 (C-9), 129.2 (C-14, 10), 113.7 (C-13, 11), 79.9 (C-5), 72.6 (C-8), 66.0 (C-7), 58.1 (C-16), 55.3 (C-15), 49.4 (C-4), 36.2 (C-2), 31.1 (C-6), 12.4 (C-17), 7.6 (C-1).

6.2.17 6-Hydroxy-2,4-dimethylheptan-3-one (51)



A flask containing LHMDS (12 mL, 12 mmol, 1M in THF) solution was treated under inert atmosphere stirring at 0 °C with a solution of ketone **50** (1 g, 10 mmol) in dry THF (2 mL), added dropwise during 15 minutes. After 30 minutes at the same temperature, a solution of epoxide **34** (232 mg, 4 mmol) in dry THF (4 mL) containing anhydrous $LiClO_4$ (637 mg, 6 mmol) was added dropwise in 10 minutes, and the resulting reaction mixture was stirred for 72 h at room temperature.[70] After dilution with water, the mixture was extracted with diethyl ether, washed with brine, dried with $MgSO_4$, filtered and concentrated to get the crude product which on further purification on flash column chromatography with 2:1 (pentane/ethyl acetate) afforded the γ-hydroxyl ketone **51** as colorless oil.

Yield: 253 mg (1.6 mmol, 40%).

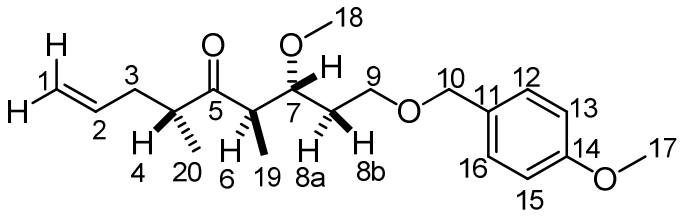
R_f = 0.18 (pentane/ethyl acetate 2:1).

EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 229(1) $[M-1]^+$, 215(7) $[M-15]^+$, 187(19), 172(12), 159(9), 145(6), 131(26), 130(5), 129(12), 117(36), 115(8), 102(5), 100(8), 75(47), 74(9), 73(100), 71(12), 69(30), 61(7), 59(7), 55(6), 47(7), 45(14), 43(41), 41(20).

^{1}H-NMR (200 MHz, CHLOROFORM-*d*) δ [ppm] = 3.89 - 4.39 (1H, m, H-6), 3.73 (1H, s, H-10), 2.94 - 3.13 (1H, m, H-4), 2.72 - 2.88 (1H, m, H-2), 2.03 - 2.16 (1H, m, H-5), 1.80 - 1.94 (1H, m, H-5), 1.15 - 1.23 (3H, m, H-7), 1.06 - 1.12 (6H, m, H-1, 9), 0.94 - 1.03 (3H, m, H-8).

^{13}C NMR (50MHz, CHLOROFORM-d) δ [ppm] = 218.6 (C-3), 65.0 (C-6), 41.8 (C-5), 40.2 (C-4), 39.2 (C-2), 23.5 (C-7), 17.7 (C-1, 9), 16.4 (C-8).

6.2.18 (4*RS*,6*RS*,7*RS*)-7-Methoxy-9-((4-methoxybenzyl)oxy)-4,6-dimethylnon-1-en-5-one (52)



To a solution of NaHMDS (0.6 mL, 1.22 mmol, 2 M in THF) in THF (0.7 mL) cooled down to -78 °C stirring under inert atmosphere, **49** (286 mg, 0.97 mmol) in THF (2 mL) was added dropwise and stirred at -78 °C for 1 h followed by the dropwise addition of allyl Iodide (0.22 mL, 2.43 mmol), stirring continued at -78 °C for 3 h. Then the mixture was allowed to warm up to room temperature overnight. The reaction mixture was quenched with saturated aqueous NH_4Cl solution at 8 °C and extracted with diethyl ether (3 x 20 mL) washed with brine, dried with $MgSO_4$, filtered, and concentrated in *vacuo*. The yellow colored crude product was purified by flash column chromatography with 5:1 pentane/diethyl ether mixture as eluent to afford the allylated product **52** as colorless oil.

Yield: 130 mg (0.4 mmol, 40%, 91% *d.e.*).

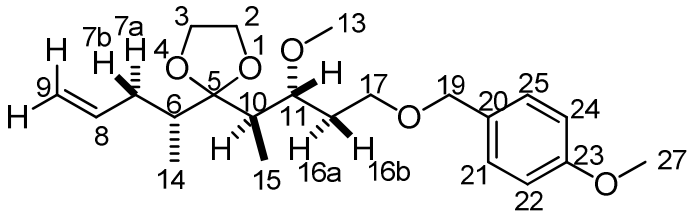
R_f = 0.55 (pentane/ethyl acetate 2:1).

EI-MS (70 eV): *m/z* (%): 334(1) $[M]^+$, 302(1), 293(1), 257(1), 246(2), 213(2), 197(1), 181(1), 177(2), 176(10), 166(2), 151(1), 137(9), 135(4), 125(3), 122(11), 121(100), 109(1), 107(1), 99(2), 97(11), 91(5), 89(2), 78(7), 77(8) 72(3), 69(21), 57(2), 55(4), 51(2), 45(2), 43(3), 41(25), 39(6).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.23 - 7.28 (2H, m, H-16, 12), 6.85 - 6.90 (2H, m, H-15, 13), 5.71 (1H, dddd, *J* = 13.6 Hz, 10.4 Hz, 6.8 Hz, 3.5 Hz, H-2), 5.05 - 5.08 (1H, m, H-1), 5.00 - 5.03 (1H, m, H-1), 4.43 (2H, d, *J* = 1.3 Hz, H-10), 3.80 (3H, s, H-17), 3.57 (1H, m, H-7), 3.53 - 3.56 (2H, m, H-9), 3.25 (3H, s, H-18), 2.90 (1H, qd, *J* = 7.1 Hz, 1.5 Hz, H-6), 2.69 (1H, sxt, *J* = 6.8 Hz, H-4), 2.34 - 2.48 (1H, m, H-3), 1.98 - 2.10 (1H, m, H-3), 1.81 - 1.93 (1H, m, H-8a), 1.59 - 1.69 (1H, m, H-8b), 1.05 (3H, d, *J* = 6.8 Hz, H-19), 0.95 (3H, d, *J* = 6.8 Hz, H-20).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 216.2 (C-5), 159.1 (C-14), 135.8 (C-2), 130.5 (C-11), 129.2 (C-16, 12), 116.7 (C-1), 113.7 (C-15, 13), 80.1 (C-7), 72.6 (C-10), 66.0 (C-9), 58.3 (C-18), 55.2 (C-17), 49.0 (C-6), 46.5 (C-4), 37.0 (C-3), 31.3 (C-8), 15.3 (C-20), 12.4 (C-19).

6.2.19 2-((2*RS*,3*RS*)-3-Methoxy-5-((4-methoxybenzyl)oxy)pentan-2-yl)-2-((*R*)-pent-4-en-2-yl)-1,3-dioxolane (54)



Allylated product **52** (30 mg, 0.091 mmol) was dissolved in ethylene glycol (0.1 mL) and stirred under inert atmosphere at room temperature. To the above solution triethyl orthoformate (0.1 mL, 0.6 mmol) and a catalytic amount of *p*-toluenesulfonic acid monohydrate (0.4 mg) was added and the mixture was stirred at room temperature for 2 h 40 min. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with chloroform (3 x 5 mL), dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by flash column chromatography with pentane/ethyl acetate mixture (gradient elution 10:1 to 2:1) to afford the dioxalane **54** as colorless oil.

Yield: 33 mg (0.087 mmol, 96%).

R_f = 0.52 (pentane/ethyl acetate 2:1).

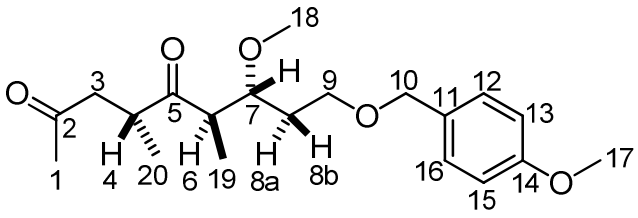
EI-MS (70 eV): *m/z* (%): 334(3) $[M-44]^+$, 302(8), 294(7), 293(30), 289(4), 257(6), 246(22), 217(9), 213(25), 209(13), 205(13), 203(6), 197(12), 195(5), 190(4), 181(29), 177(42), 176(87), 175(22), 169(16), 166(34), 165(9), 161(6), 151(16), 149(15), 145(13), 138(28),

137(83), 136(50), 135(64), 129(19), 125(38), 123(26), 122(84), 121(100), 119(15), 111(12), 109(27), 107(25), 106(21), 99(31), 97(82), 94(16), 91(47), 89(27), 87(15), 78(55), 77(59), 73(33), 72(35), 69(84), 67(24), 65(18), 55(23), 53(16), 51(10).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.16 - 7.22 (2H, m, H-21, 25), 6.77 - 6.83 (2H, m, H-24, 22), 5.59 - 5.70 (1H, m, *J* = 14.2 Hz, 9.7 Hz, 7 Hz, H-8), 4.98 - 5.02 (1H, m, H-9), 4.93 - 4.96 (1H, m, H-9), 4.36 (2H, s, H-19), 4.00 - 4.06 (2H, m, H-2), 3.85 - 3.90 (2H, m, H-3), 3.73 (3H, s, H-27), 3.52 - 3.56 (2H, t, *J* = 7.2 Hz, H-17), 3.37 - 3.51(1H, m, H-11), 3.18(3H, s, H-13), 2.77 - 2.91 (1H, m, H-7b), 2.57 - 2.70 (1H, m, H-10), 2.26 - 2.40 (1H, m, H-7a), 1.92 - 2.03 (1H, dqd, *J* = 14.4 Hz, 7.4 Hz, 2.5 Hz, H-6), 1.75 - 1.87 (1H, m, H-16a), 1.50 - 1.60 (1H, m, H-16b), 0.99 (3H, s, H-14), 0.89 (3H, s, H-15).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 159.1 (C-23), 135.8 (C-8), 130.5 (C-20), 129.2 (C-25, 21), 116.7 (C-5), 115.1 (C-9), 113.7 (C-24, 22), 80.1 (C-11), 73.0 (C-19), 66.0 (C-17), 63.9 (C-2, 3), 58.3 (C-13), 55.2 (C-27), 49.0 (C-10), 46.4 (C-6), 36.2 (C-7), 31.3 (C-16), 15.3 (C-14), 12.4 (C-15).

6.2.20 (4*RS*,6*RS*,7*RS*)-7-Methoxy-9-((4-methoxybenzyl)oxy)-4,6-dimethylnonane-2,5-dione (53)



Anhydrous palladium chloride (6 mg, 0.034 mmol) and anhydrous copper acetate (21 mg, 0.11 mmol) were suspended in a mixture of DMF (4 mL) and water (0.5 mL) and O_2 was bubbled through the reaction mixture at atmospheric pressure and stirred for 30 min. The addition of terminal alkene **52** (51 mg, 0.15 mmol) dissolved in DMF (1.5 mL) followed and the mixtrure was stirred for 4 d at room temperature. The reaction mixture was diluted with water and extracted with diethyl ether (3 x 10 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with pentane/ethyl acetate mixture (gradient elution 10:1 to 2:1) to afford the diketone **53** as colorless oil.

Yield: 27 mg (0.11 mmol, 50%).

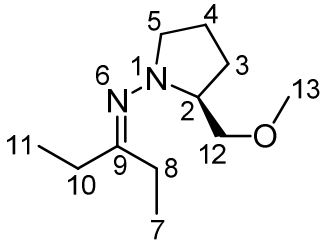
R_f = 0.20 (pentane/ethyl acetate 2:1).

EI-MS (70 eV): *m/z* (%): 350(1) $[M]^+$, 332(1), 318(1), 274(1), 260(1), 209(1), 205(1), 182(5), 181(2), 177(3), 176(17), 152(2), 137(9), 136(3), 135(6), 124(7), 122(12), 121(100), 113(15), 109(3), 107(2), 97(4), 91(5), 89(3), 85(3), 78(8), 77(9), 73(3), 71(3), 69(5), 65(2), 58(3), 55(3), 51(2), 45(2), 43(31), 41(5), 39(2).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.24 - 7.28 (2H, m, H-16, 12), 6.86 - 6.90 (2H, m, H-15, 13), 4.43 (2H, s, H-10), 3.80 (3H, s, H-17), 3.59 (1H, ddd, *J* = 8.3 Hz, 7.1 Hz, 3.3 Hz, H-7), 3.55 (2H, t, *J* = 6.1 Hz, H-9), 3.24 (3H, s, H-18), 3.07-3.16 (1H, m, H-4), 3.01 (1H, dd, *J* = 16.4 Hz, 6.1 Hz, H-3), 2.91-2.98 (1H, m, H-6), 2.34 (1H, m, *J* = 8.6 Hz, 6.8 Hz, H-3), 2.14 (3H, s, H-1), 1.88 (1H, s, H-8b), 1.55 - 1.67 (1H, m, H-8a), 1.08 - 1.12 (3H, m, H-20), 1.02 - 1.07 (3H, m, H-19).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 215.7 (C-5), 207.3 (C-2), 159.1 (C-14), 130.6 (C-11), 129.2 (C-16, 12), 113.7 (C-15, 13), 79.4 (C-7), 72.6 (C-10), 66.0 (C-9), 58.4 (C-18), 55.3 (C-17), 49.2 (C-6), 46.3 (C-3), 41.0 (C-4), 31.1 (C-8), 30.1 (C-1), 15.8 (C-20), 13.0 (C-19).

6.2.21 (*S*)-2-(Methoxymethyl)-*N*-(pentan-3-ylidene)pyrrolidin-1-amine (59)



To the flask containing SAMP **58a** (1 g, 7.68 mmol) equipped with reflux condenser and drying tube, diethylketone **31** (660 mg, 7.68 mmol) was added dropwise and heated to 60 °C for 21 h monitored by TLC.[78] The mixture was poured into a solution containing dichloromethane/water 6:1. The organic phase dried with $MgSO_4$, filtered, and concentrated in *vacuo* to afford the light yellow colored oil **59** after quick filtration over a short pad of silica gel.

Yield: 1.52 g (7. 68 mmol, quant.).

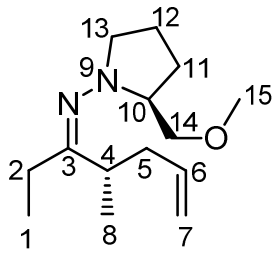
R_f = 0.20 (pentane/diethyl ether 1:1).

EI-MS (70 eV): *m/z* (%): 199(8) $[M+1]^+$, 198(43) $[M]^+$, 154(53), 153(99), 137(3), 114(5), 113(12), 112(4), 98(10), 85(8), 84(47), 83(10), 82(18), 80(6), 58(10), 57(32), 56(100), 55(54), 54(31), 53(22), 52(6), 51(5), 45(70), 43(27), 42(52), 41(76), 40(19), 39(51).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 3.36 - 3.42 (1H, m, H-12), 3.34 (3H, s, H-13), 3.21 (1H, ddd, *J* = 9.3 Hz, 6.8 Hz, 2.5 Hz, H-2), 3.00 - 3.16 (2H, m, H-12, H-5), 2.34 - 2.53 (3H, m, H-5, 8), 2.17 - 2.31 (2H, m, H-10), 1.93 - 2.04 (1H, m, H-3), 1.76 - 1.88 (2H, m, H-4), 1.59 - 1.73 (1H, m, H-3), 1.10 (3H, t, *J* = 8.0 Hz, H-11), 1.05 (3H, t, *J* = 7.6 Hz, H-7).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 173.5 (C-9), 75.4 (C-12), 66.0 (C-2), 59.1 (C-13), 55.0 (C-5), 28.7 (C-10), 26.5 (C-3), 23.4 (C-8), 21.9 (C-4), 11.8 (C-11), 10.9 (C-7).

6.2.22 (*S,Z*)-2-(Methoxymethyl)-*N*-((*S*)-4-methylhept-6-en-3-ylidene)pyrrolidin-1-amine (60)



***Preparation of LDA solution*:**

n-BuLi (7.2 mL, 11.51 mmol, 1.6 M in hexane) was slowly added dropwise to the diisopropylamine (1.6 mL, 11.59 mmol) in dry diethyl ether (23 mL) at -60 °C and stirred for 10 minutes warmed up to room temperature under water bath. The flask was cooled down to 0 °C under ice bath and stirred for 1 h observing the formation of the light yellow colored solution.

Compound **59** (1.52 g, 7.68 mmol) was added slowly dropwise to the freshly prepared LDA solution stirring at 0 °C under inert atmosphere. The resulting yellow colored solution slowly solidified into a semi solid and was stirred for 4 h at 0 °C. The reaction mixture was cooled down to -110 °C followed by the dropwise addition of allyl iodide **33** (1.42 g, 8.44 mmol) in diethyl ether (3 mL), stirred for 3 h and allowed to warm up to room temperature overnight.[78,125] The reaction mixture was poured into 2:1 diethyl ether/water mixture and the

aqueous layer washed with diethylether (2 x 30 mL). The combined organic layers were washed with water, dried with $MgSO_4$, filtered, and concentrated in *vacuo* to get the yellow colored crude product. Purification of the crude product was done by flash column chromatography with 2:1 pentane/diethyl ether mixture as eluent afforded alkene **60** as light yellow colored oil.

Yield: 1.92 g (8.08 mmol, 95%, 99% *d.e.*).

$[\alpha]_D^{21.5}$ = +140.8 (*c* 2.31, Et_2O).

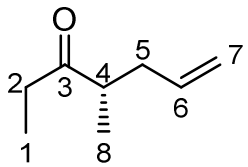
R_f = 0.41 (pentane/diethyl ether 1:1).

EI-MS (70 eV): *m/z* (%): 238(1) $[M]^+$, 223(1), 209(1), 194(13), 193(77), 153(3), 151(4), 142(2), 136(2), 125(23), 124(25), 97(6), 96(26), 84(10), 82(13), 70(12), 69(73), 69(100), 68(20), 67(17), 45(38), 42(21), 41(99), 39(28).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 5.60 - 5.88 (1H, m, H-6), 4.92 - 5.09 (2H, m, H-7), 3.34 - 3.41 (1H, m, H-14), 3.33 - 3.34 (3H, s, H-15), 3.16 - 3.24 (1H, m, H-10), 2.95 - 3.14 (2H, m, H-13, 14), 2.39 - 2.48 (2H, m, H-4, 13), 2.32 (2H, q, *J* = 7.8 Hz, H-2), 2.05 - 2.21 (2H, m, H-5), 1.95 - 2.04 (1H, m, H-11), 1.76 - 1.88 (2H, m, H-12, 11), 1.59 - 1.72 (1H, m, H-12), 1.09 (2H, t, *J* = 7.8 Hz, H-1), 1.05 (3H, d, *J* = 6.8 Hz, H-8).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 174.8 (C-3), 137.3 (C-6), 115.6 (C-7), 75.4 (C-14), 66.0 (C-10), 59.1 (C-15), 54.8 (C-13), 39.6 (C-4), 38.9 (C-5), 26.5 (C-11), 23.4 (C-2), 21.9 (C-12), 19.0 (C-8), 11.0 (C-1).

6.2.23 (*S*)-4-Methylhept-6-en-3-one (61)



NaH/MeI Salt method:[78]

To the flask containing **60** (94 mg, 0.40 mmol), iodomethane (282 mg, 2 mmol) was added and heated to reflux at 65 °C for 2 d. After cooling down to room temperature, 4 N HCl (2 mL) (50 mL/10 mmol) was added and the mixure stirred for 5 min. The contents of the flask

were transferred into a Erlenmeyer flask followed by the addition of pentane (10 mL) and stirred vigorously for 30 minutes. The organic phase were separated, washed with saturated solution of $NaHSO_3$ solution and pH 7 buffer solution, dried with Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 5:1 pentane/diethyl ether mixture to afford ketone **61** as the colorless volatile fruity odour liquid.

Yield: 30 mg (0.24 mmol, 60%, 90% *ee*).

***Copper Chloride cleavage method*:**[79]

To the ice cooled solution of **60** (136 mg, 0.57 mmol) in THF (6 mL), copper chloride 1M aqueous solution (1 mL) was added dropwise and stirred for 1 d, monitored by TLC. After the consumption of the starting material aqueous NH_3 (25% aq. 3 mL) was added dropwise and extracted with diethyl ether (3 x 10 mL). The organic phases combined washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 5:1 pentane/diethyl ether mixture to afford the chiral ketone **61** as colorless volatile fruity odour liquid.

yield: 65 mg (0.51 mmol, 90%, 60% *ee*).

Lipodex-G column, Injection mode: splitless; Temperature program: 50 °C for 1 min, then with 1 °C/min up to 210 °C; R_t: 12.46 min.(minor), 13.10 min.(major).

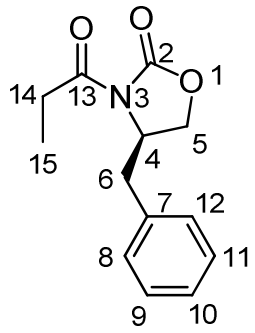
R_f = 0.64 (pentane/diethyl ether 1:1).

EI-MS (70 eV): *m/z* (%): 126(8) $[M]^+$, 97(18), 69(72), 67(10), 57(100), 55(8), 53(11), 43(7), 41(76), 40(6), 39(38).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 5.72 (1H, ddt, *J* = 17.1 Hz, 10.0 Hz, 7.0 Hz, H-6), 4.99 - 5.06 (2H, m, H-7), 2.61 (1H, dq, *J* = 13.6 Hz, 6.8 Hz, H-4), 2.46 (2H, q, *J* = 7.1 Hz, H-2), 2.35 - 2.42 (1H, m, *J* = 14.1 Hz, 7.3 Hz, 1.5 Hz, H-5), 2.09 (1H, dtt, *J* = 14.2 Hz, 7.2 Hz, 1.3 Hz, H-5), 1.08 (3H, d, *J* = 6.8 Hz, H-8), 1.04 (3H, t, *J* = 7.3 Hz, H-1).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 214.1 (C-3), 135.5 (C-6), 116.3 (C-7), 45.4 (C-4), 36.9 (C-5), 34.1 (C-2), 15.8 (C-8), 7.3 (C-1).

6.2.24 (*R*)-4-Benzyl-3-propionyloxazolidin-2-one (65)



To the solution of (*R*)-4-benzyl-2-oxazolidinone **64** (767 mg, 4.33 mmol) in 35 mL anhydrous THF at -78 °C *n*-BuLi (2.9 mL, 4.72 mmol, 1.6 M in hexane)was added over a period of 15 min dropwise and stirred under inert atmosphere for 30 min followed by the dropwise addition of the propionyl chloride **63** (437 mg, 4.72 mmol) *via* a syringe for 15 min. The reaction mixture was allowed to warm up to room temperature for 3 h and stirred at 0 °C for 3 h followed by the quenching with saturated aqueous NH_4Cl solution. The quenched reaction mixture was concentrated in vacuo and the residue extracted with dichloromethane. The organic layers were washed with 10 % aqueous NaOH solution and the aqueous layer was extracted with dichloromethane. The combined organic layers were washed with brine solution, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product as light colored oil. Purification by flash chromatography with 5:1 pentane/ethyl acetate mixture as eluent afforded the desired product **65** as a white needles.[84]

Yield: 1 g (4.3 mmol, 99%, 99% *ee*).

$[\alpha]_D^{20}$ = -102 (*c* 1.0, EtOH).

Mp = 44-46 °C

R_f = 0.56 (pentane/ethyl acetate 5:1).

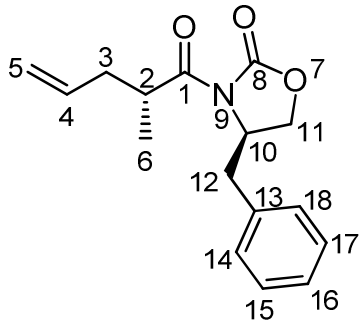
EI-MS (70 eV): *m/z* (%): 234(2) $[M+1]^+$, 233(15) $[M]^+$, 148(5), 142(18), 134(4), 133(4), 117(9), 116(7), 115(9), 103(2), 92(10), 91(42), 90(4), 89(7), 86(7), 78(4), 77(7), 65(22), 64(2), 63(7), 58(6), 57(100), 56(3), 55(4), 52(3), 51(9), 50(3), 42(9), 41(5), 40(2), 39(11).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.30 - 7.37 (2H, m, H-9, 11), 7.24 - 7.30 (1H, m, H-10), 7.18 - 7.23 (2H, m, H-8, 12), 4.67 (1H, dddd, *J* = 10.4 Hz, *J* = 9.7 Hz, *J* = 7.0

Hz, *J* = 3.4 Hz, H-4), 4.17 (2H, ddd, *J* = 9.1 Hz, *J* = 5.9 Hz, *J* = 3.6 Hz, H-5), 3.30 (1H, dd, *J* = 13.3 Hz, *J* = 3.2 Hz, H-6), 2.96 (2H, dqd, *J* = 9.3 Hz, *J* = 7.4 Hz, *J* = 1.3 Hz, H-14), 2.78 (1H, dd, *J* = 9.7 Hz, *J* = 3.6 Hz, H-6), 1.21 (3H, t, *J* = 7.4 Hz, H-15).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 174.0 (C-13), 153.5 (C-2), 135.3 (C-7), 129.4 (C-8, 12), 128.9 (C-9, 11), 127.3 (C-10), 66.2 (C-5), 55.1 (C-4), 37.9 (C-6), 29.2 (C-14), 8.3 (C-15).

6.2.25 (*R*)-4-Benzyl-3-((*R*)-2-methylpent-4-enoyl)oxazolidin-2-one (66)



Compound **65** (1 g, 4.3 mmol) was dissolved in anhydrous THF (11 mL) while stirring under inert atmosphere at -78 °C. NaHMDS (3.4 mL, 6.85 mmol, 2 M in THF) along with anhydrous THF (3.2 mL) was added dropwise and allowed to stir for 1 h at -78 °C. Allyl bromide (1.8 mL, 21 mmol) was added dropwise to the reaction mixture at -78 °C for 10 min and allowed to warm up to -45 °C. The reaction mixture was stirred at -45 °C for 4 h, followed by quenching with saturated NH_4Cl solution. The aqueous phase was extracted twice with ethyl acetate and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo to obtain the crude product as light yellow colored oil. Purification of the crude product with flash column chromatography using 10:1 pentane/ethyl acetate as eluent afforded the desired product **66** as colorless oil.[126]

Yield: 1.1 g (4.0 mmol, 94%, 99% *d.e.*).

$[\alpha]_D^{21}$ = -41.5 (*c* 0.9, $CHCl_3$) , $[\alpha]_D^{19}$ = -39 (*c* 1, $CHCl_3$), lit.[127]

R_f = 0.23 (pentane/diethyl ether 2:1).

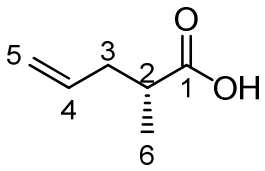
EI-MS (70 eV): *m/z* (%): 274(3) $[M+1]^+$, 273(13) $[M]^+$, 187(3), 181(7), 178(10), 139(2), 134(3), 132(5), 129(2), 127(3), 118(5), 117(34), 116(10), 115(18), 103(4), 98(6), 97(85),

96(17), 95(5), 92(21), 91(82), 90(4), 89(7), 86(22), 81(3), 78(5), 77(8), 70(8), 69(100), 68(20), 67(23), 66(3), 65(22), 63(3), 57(3), 56(5), 55(7), 54(4), 53(12), 52(3), 51(5).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.30 - 7.37 (2H, m, *J* = 7.2 Hz, H-15, 17), 7.26 - 7.30 (1H, m, *J* = 8.0 Hz, *J* = 6.8 Hz, H-16), 7.19 - 7.25 (2H, m, *J* = 8.1 Hz, H-14, 18), 5.82 (1H, ddt, *J* = 17.0 Hz, *J* = 10.0 Hz, *J* = 7.0 Hz, H-4), 5.03 - 5.15 (2H, m, *J* = 16.1 Hz, *J* = 9.1 Hz, *J* = 5.9 Hz, H-5), 4.68 (1H, ddt, *J* = 9.8 Hz, *J* = 7.0 Hz, *J* = 3.4 Hz, H-10), 4.12 - 4.23 (2H, m, *J* = 9.1 Hz, *J* = 7.2 Hz, *J* = 3.6 Hz, H-11), 3.87 (1H, q, *J* = 6.8 Hz, H-2), 3.29 (1H, dd, *J* = 13.3 Hz, *J* = 3.2 Hz, H-12), 2.70 (1H, dd, *J* = 13.4 Hz, *J* = 9.8 Hz, H-12), 2.53 (1H, dtt, *J* = 13.8 Hz, *J* = 6.8 Hz, *J* = 1.3 Hz, H-3), 2.24 (1H, dtt, *J* = 13.9 Hz, *J* = 7.0 Hz, *J* = 1.2 Hz, H-3), 1.19 (3H, d, *J* = 6.8 Hz, H-6).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 176.5 (C-1), 153.1 (C-8), 135.4 (C-4), 135.2 (C-13), 129.4 (C-14, 18), 128.9 (C-15, 17), 127.3 (C-16), 117.2 (C-5), 66.0 (C-11), 55.4 (C-10), 38.1 (C-3), 38.0 (C-12), 37.1 (C-2), 16.4 (C-6).

6.2.26 (*R*)-2-Methylpent-4-enoic acid (67)



To a solution of **66** (500 mg, 1.83 mmol) dissolved in 37 mL of 3:1 THF/H_2O 30 % at 0 °C under stirring aqueous H_2O_2 (1.12 mL, 11 mmol) and LiOH monohydrate (88 mg, 3.6 mmol) were added portion wise and allowed to stir at 0 °C for 2 h. The reaction was controlled by TLC. The reaction mixture was quenched by the careful and slow addition of 2 M solution of aqueous $Na_2S_2O_3$ after the consumption of the starting material. This was allowed to stir further for 20 min at 0 °C. The THF was evaporated in the rotavapor and the residue was extracted with dichloromethane 4 to 5 times. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to obtain the chiral auxiliary as light colored oil. Purification of the crude chiral auxiliary with pentane/ethyl acetate afforded **64** as colorless crystals.[84,86,128]

To the aqueous layer at 0 °C a saturated solution of NaH_2PO_4. H_2O was added.[129] The reaction mixture was acidified at 0 °C to pH 1-3 with 1N HCl solution, followed by the extraction with diethyl ether five times. The combined organic layers were dried with $MgSO_4$,

filtered, and concentrated under reduced pressure to obtain **67** as light yellow colored crude liquid. Purification of the crude product by flash column chromatography with 2:1 pentane/diethyl ether mixture as eluent afforded the desired product **67** as colorless pungent odor liquid.

yield: 206 mg (1.81 mmol, 99%, 98% *ee*).

MSTFA derivative: Lipodex-G column; Injection mode: split ratio 20:1; Temperature program: 50 °C for 5 min, then with 10 °C/min up to 210 °C; R_t: 70.97 min.(minor), 72.40 min.(major).

$[\alpha]_D^{21}$ = +28.75 (*c* 2.0, $CHCl_3$).

R_f = 0.46 (pentane/diethyl ether 1:1).

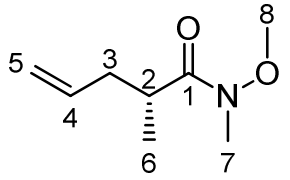
EI-MS (70 eV): *m/z* (%): 114(6) $[M]^+$, 99(16), 81(3), 74(4), 73(5), 71(6), 70(8), 69(85), 68(15), 67(13), 58(2), 56(11), 55(15), 54(4), 53(14), 51(4), 50(3), 45(17), 44(4), 43(11), 42(14), 41(100), 39(51), 37(3).

EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 186(3) $[M]^+$, 171(14), 81(2), 77(3), 76(5), 75(67), 74(10), 73(100), 69(4), 68(11), 67(4), 61(6), 59(4), 58(2), 55(3), 53(3), 47(11), 45(23), 44(2), 43(7), 42(2), 41(22), 39(11).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 5.77 (1H, ddt, *J* = 16.7 Hz, *J* = 10.2 Hz, *J* = 6.8 Hz, H-4), 5.03 - 5.13 (2H, m, H-5), 2.56 (1H, q, *J* = 7.0 Hz, H-2), 2.45 (1H, ddd, *J* = 13.6 Hz, *J* = 6.8 Hz, *J* = 4.2 Hz, H-3), 2.21 (1H, ddd, *J* = 14.2 Hz, *J* = 9.5 Hz, *J* = 6.8 Hz, H-3), 1.19 (3H, d, *J* = 7.0 Hz, H-6).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 182.1 (C-1), 135.1 (C-4), 117.2 (C-5), 39.0 (C-2), 37.4 (C-3), 16.3 (C-6).

6.2.27 (*R*)-*N*-Methoxy-*N*,2-dimethylpent-4-enamide (70)



To the solution of **67** (126 mg, 1.1 mmol) in dichloromethane (5 mL) stirring at room temperature under inert atmosphere 1,1'-carbonyldiimidazole **68** (179 mg, 1.1 mmol) was added and allowed to stir for 30 min. To the reaction mixture *N,O*-dimethylhyxdroxylamine hydrochloride **69** (108 mg, 1.1 mmol) was added in one portion and stirred overnight for approx. 20 h, monitored by the TLC for the consumption of the acid. The reaction mixture was diluted with dichloromethane and the combined organic layers washed with 0.25 M HCl solution, saturated $NaHCO_3$ solution, brine solution, dried with $MgSO_4$, filtered and concentrated under reduced pressure to obtain crude amide **70** as fruity odor colored liquid. Purification of the crude product with flash column chromatography using gradient elution from 10:1 to 3:1 pentane/diethyl ether mixture as eluent afforded the desired amide **70** as fruity odor colorless liquid.[130]

Yield: 174 mg (1.1 mmol, 99%, 99% *ee*).

Lipodex-G column; Injection mode: split ratio 20:1; Temperature program: 50 °C for 1 min, then with 1 °C/min up to 210 °C; R_t: 32.71 min.(minor), 33.99 min.(major).

$[\alpha]_D^{20}$ = +12.41 (*c* 1.16, $CHCl_3$).

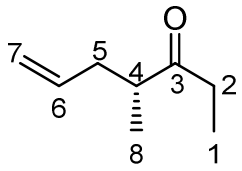
R_f = 0.27 (pentane/diethyl ether 1:1).

EI-MS (70 eV): *m/z* (%): 142(1) $[M]^+$, 127(3), 126(7), 112(4), 98(3), 97(35), 96(2), 88(2), 81(1), 73(1), 70(8), 69(100), 68(5), 67(10), 66(1), 65(3), 62(2), 61(14), 60(5), 58(5), 56(7), 55(7), 54(3), 53(10), 51(2), 50(1), 46(4), 45(4), 43(3), 42(10), 41(86), 40(4), 39(24), 38(2).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 5.70 (1H, ddt, *J* = 16.7 Hz, *J* = 10.2 Hz, *J* = 7.0 Hz, H-4), 4.88 - 5.05 (2H, m, H-5), 3.62 (3H, s, H-8), 3.12 (3H, s, H-7), 2.90 (1H, dqd, *J* = 9.1 Hz, *J* = 6.6 Hz, *J* = 4.4 Hz, H-2), 2.36 (1H, dddt, *J* = 14.0 Hz, *J* = 7.5 Hz, *J* = 6.5 Hz, *J* = 1.3 Hz, H-3), 2.05 (1H, dddt, *J* = 14.0 Hz, *J* = 7.0 Hz, *J* = 4.4 Hz, *J* = 2.3 Hz, H-3), 1.06 (3H, d, *J* = 7.0 Hz, H-6).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 177.3 (C-1), 136.2 (C-4), 116.4 (C-5), 61.5 (C-8), 37.8 (C-3), 36.1 (C-2), 29.7 (C-7), 17.0 (C-6).

6.2.28 (*R*)-4-Methylhept-6-en-3-one (61)



To a stirred solution of **70** (100 mg, 0.64 mmol) in anhydrous THF (2 mL) at -20 °C under inert atmosphere ethyl magnesium bromide **71** (0.4 mL, 0.96 mmol, 3 M in diethyl ether) was added dropwise. Stirring was continued at -20 °C until the consumption of the amide was detected by TLC. The reaction mixture was quenched by slow addition of saturated NH_4Cl solution and extracted three to five times with diethyl ether. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated in rotavapor to obtain the crude chiral allyl ketone. Purification of the crude product by flash chromatography with 5:1 pentane/diethyl ether mixture as eluent afforded (*R*)-chiral allyl ketone **61** as fruity odor colorless volatile liquid.[88]

Yield: 72 mg (0.57 mmol, 90%, 99% *ee*).

Lipodex-G column; Injection mode: split ratio 20:1; Temperature program: 50 °C for 1 min, then with 1 °C/min up to 210 °C; R_t: 12.46 min.(minor), 13.10 min.(major).

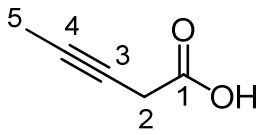
R_f = 0.64 (pentane/diethyl ether 1:1).

EI-MS (70 eV): *m/z* (%): 127(3) $[M+1]^+$, 126(24) $[M]^+$, 111(8), 108(2), 99(3), 98(4), 97(42), 93(2), 85(2), 84(3), 83(5), 79(4), 77(3), 70(11), 69(90), 68(13), 67(22), 65(5), 63(2), 58(10), 57(100), 56(9), 55(15), 54(7), 53(18), 52(3), 51(6), 50(3), 43(11), 42(10), 41(76), 40(6), 39(41), 38(3).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 5.72 (1H, ddt, *J* = 17.1 Hz, 10.0 Hz, 7.0 Hz, H-6), 4.99 - 5.06 (2H, m, H-7), 2.61 (1H, dq, *J* = 13.6 Hz, 6.8 Hz, H-4), 2.46 (2H, q, *J* = 7.1 Hz, H-2), 2.35 - 2.42 (1H, m, *J* = 14.1 Hz, 7.3 Hz, 1.5 Hz, H-5), 2.09 (1H, dtt, *J* = 14.2 Hz, 7.2 Hz, 1.3 Hz, H-5), 1.08 (3H, d, *J* = 6.8 Hz, H-8), 1.04 (3H, t, *J* = 7.3 Hz, H-1).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 214.1 (C-3), 135.5 (C-6), 116.3 (C-7), 45.4 (C-4), 36.9 (C-5), 34.1 (C-2), 15.8 (C-8), 7.3 (C-1).

6.2.29 Pent-3-ynoic acid (73)



To the solution of CrO_3 (3 g, 30 mmol) in diluted solution containing concentrated H_2SO_4 (2 mL) and water (7.6 mL) under ice bath (-5 to 0 °C), pent-3-yn-1-ol **72** (1 g, 11.9 mmol) in acetone (119 mL) was added slowly dropwise via a dropping funnel. The reaction mixture was allowed to stir at room temperature for 15 h. The reaction was monitored by TLC and quenched with isopropanol to destroy excess Jones reagent. The mixiture was filtered over a pad of celite and washed with diethyl ether. The organic extract was washed with brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Purification of the crude product by flash column chromatography using 20:1 dichloromethane/methanol mixture as eluent afforded the desired acid **73** as white crystalline powder.[91,92]

Yield: 695 mg (6.7 mmol, 60%).

R_f = 0.29 (dichloromethane/methanol 20:1).

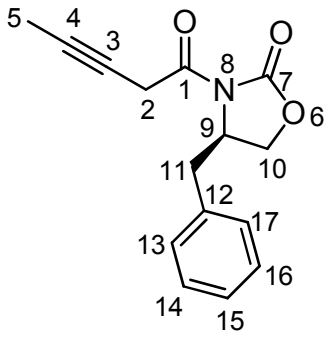
EI-MS (70 eV): *m/z* (%): 98(22) $[M]^+$, 70(6), 69(4), 55(5), 54(49), 53(84), 52(39), 51(81), 50(100), 49(30), 48(5), 45(78), 44(11), 43(2), 42(12), 41(9), 40(4), 39(54), 38(17), 37(23), 36(6).

EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 155(35) $[M-15]^+$, 128(4), 127(12), 126(14), 117(7), 111(25), 99(11), 83(12), 75(40), 73(100), 61(6), 59(7), 53(34), 51(21), 50(16), 47(7), 45(34), 43(16), 41(5).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 10.59 (1H, br. s., -COO*H*), 3.32 (2H, s, H-2), 1.84 (3H, s, H-5).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 175.4 (C-1), 79.9 (C-4), 69.7 (C-3), 25.8 (C-2), 3.7 (C-5).

6.2.30 (*R*)-4-Benzyl-3-(pent-3-ynoyl)oxazolidin-2-one (75)



To the solution of pent-3-ynoic acid **73** (654 mg, 6.66 mmol) in freshly distilled THF (36 mL) under inert atmosphere at -78 °C, Et_3N (809 mg, 7.99 mmol) and Pivaloyl chloride (**74**) (965 mg, 8 mmol) was added dropwise. The resulting mixture was stirred for 20 min at -78 °C, warmed to 0 °C, and allowed to stir for 45 min at this temperature. Meanwhile, to the solution of (*R*)-4-benzyl-2-oxazolidinone **64** (1.18 g, 8 mmol) in freshly distilled THF (29 mL) at -80 °C under inert atmosphere, *n*-BuLi (5 mL, 8 mmol, 1.6 M in hexane) was added dropwise. The resultant solution was transferred via cannula to the mixed anhydride, allowed to stir for 15 min, warmed to 0 °C within 3 h and stirred for 45 min at 0 °C. After quenching with saturated NH_4Cl solution, the mixture was extracted 5 times with TBME. The combined organic extracts were washed with water and dried with $MgSO_4$. The solution was filtered and concentrated in vacuo. Purification of the crude product by flash chromatography using gradient elution 10:1 to 2:1 pentane/TBME mixture as eluent afforded the product **75** as colorless solid.[93]

Yield: 616 mg (2.4 mmol, 36%, 99% *ee*).

R_f = 0.41 (pentane/TBME 1:1).

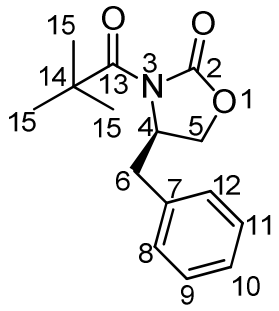
EI-MS (70 eV): *m/z* (%): 258(8) $[M+1]^+$, 257(40) $[M]^+$, 256(33), 178(13), 166(6), 128(5), 122(36), 118(6), 117(39), 116(8), 115(23), 103(7), 92(20), 91(100), 90(6), 89(13), 86(22), 81(15), 80(20), 79(8), 78(12), 77(17), 65(39), 63(13), 58(6), 53(49), 52(36), 51(40), 50(18), 43(10), 42(14), 41(11), 39(23).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.16 - 7.22 (2H, m, H-14, 16), 7.09 - 7.16 (1H, m, H-15), 7.03 - 7.08 (2H, m, H-13, 17), 4.53 (1H, dddd, *J* = 9.5 Hz, *J* = 7.2 Hz, *J* = 6.8 Hz, *J* = 3.2 Hz, H-9), 4.00 - 4.12 (2H, m, H-10), 3.74 (2H, quin, *J* = 2.4 Hz, H-2), 3.18 (1H,

dd, *J* = 13.4 Hz, *J* = 3.2 Hz, H-11), 2.63 (1H, dd, *J* = 13.3 Hz, *J* = 9.6 Hz, H-11), 1.73 (3H, t, *J* = 2.5 Hz, H-5).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 168.3 (C-1), 153.1 (C-7), 135.0 (C-12), 129.4 (C-13, 17), 129.0 (C-14, 16), 127.4 (C-15), 80.4 (C-4), 69.9 (C-3), 66.4 (C-10), 55.4 (C-9), 37.6 (C-11), 28.0 (C-2), 3.7 (C-5).

6.2.31 (*R*)-4-Benzyl-3-pivaloyloxazolidin-2-one (76)

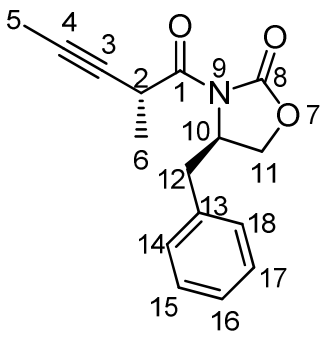


EI-MS (70 eV): *m/z* (%): 261(7) $[M]^+$, 204(6), 170(12), 142(6), 117(15), 116(8), 115(16), 92(16), 91(84), 90(8), 89(14), 86(27), 85(14), 78(4), 77(10), 70(5), 69(8), 65(36), 63(8), 58(7), 57(100), 56(14), 55(12), 53(4), 51(9), 42(16), 41(85), 40(9), 39(41).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.32 - 7.36 (1H, m, H-10), 7.25 - 7.31 (2H, m, H-9, 11), 7.19 - 7.24 (2H, m, H-8, 12), 4.70 (1H, ddt, *J* = 9.6 Hz, *J* = 7.3 Hz, *J* = 3.2 Hz, H-4), 4.11 - 4.24 (2H, m, H-5), 3.23 (1H, dd, *J* = 13.4 Hz, *J* = 3.4 Hz, H-6), 2.77 (1H, dd, *J* = 13.3 Hz, *J* = 9.6 Hz, H-6), 1.40 (9H, s, H-15).

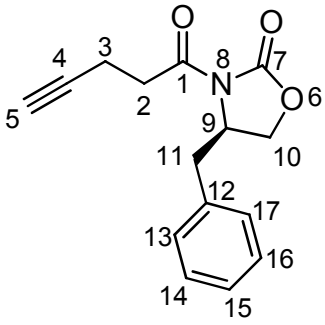
^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 178.5 (C-13), 152.3 (C-2), 135.5 (C-7), 129.4 (C-8, 12), 128.8 (C-9, 11), 127.2 (C-10), 66.1 (C-5), 57.4 (C-4), 41.7 (C-14), 37.8 (C-6), 26.3 (C-15).

6.2.32 (*R*)-4-Benzyl-3-((*R*)-2-methylpent-3-ynoyl)oxazolidin-2-one (77)



To the solution of **75** (627 mg, 2.44 mmol) in freshly distilled THF (8 mL) under inert atmosphere at -78 °C, NaHMDS (4.6 mL, 4.6 mmol, 1 M in THF) was added dropwise using a syringe pump over 20 min and allowed to stir for 45 min at -78 °C. Methyl iodide (0.8 mL, 12.2 mmol) was added slowly dropwise and stirred for 5 h at -78 °C, quenched with saturated NH_4Cl solution, water and extracted five times with TBME. The combined organic extracts were washed with water and brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography with 5:1 pentane/ethyl acetate mixture as eluent afforded the desired product **77** as colorless oil with the isomerized product from internal alkyne to terminal alkyne.

6.2.33 (*R*)-4-Benzyl-3-(pent-4-ynoyl)oxazolidin-2-one (82)



To a stirred solution of pent4ynoic acid **81** (1 g, 10 mmol) in freshly distilled THF (100 mL) at -78 °C under inert atmosphere, Et_3N (1.5 mL, 10.5 mmol) was added, followed by the dropwise addition of pivaloyl chloride (**74**) (1.3 mL, 10.5 mmol). The resulting mixture stirred at -78 °C for 15 min and allowed to warm to room temperature within 4 hours. The reaction mixture was further stirred for 30 min at room temperature and re-cooled to -78 °C. Mean while, a solution of (*R*)-4-benzyl-2-oxazolidinone **64** (1.77 g, 10 mmol) in freshly distilled THF (50 mL) was cooled to -85 °C and treated slowly with a 1.6 M solution of *n*-BuLi(6.8 mL, 11

mmol). The resulting solution was stirred at -78 °C for 15 min and then added via cannula into the solution of the mixed anhydride. The reaction mixture was stirred for 30 min at -78 °C and allowed to warm up to room temperature followed by quenching with saturated NH_4Cl solution and extracted with ethyl acetate. The combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain the crude product as dark brown colored oil. Purification of the crude product by flash column chromatography using gradient elution 20:1 to 4:1 pentane/ethyl acetate mixture as eluent afforded the desired product **82** as white solid.[94]

Yield: 2.4 g (9.4 mmol, 94%, 99% *ee*).

M.pt. = 87-90 °C.

$[\alpha]_D^{20}$ = -98.4 (*c* 1.8 dichloromethane).

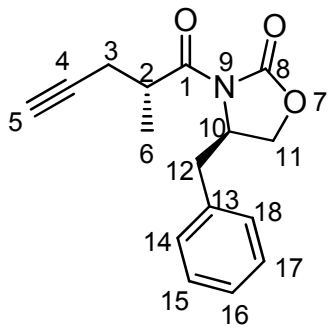
R_f = 0.32 (pentane/ethyl acetate 2:1).

EI-MS (70 eV): *m/z* (%): 258(2) $[M+1]^+$, 257(12) $[M]^+$, 256(5), 172(8), 166(18), 156(3), 140(4), 134(5), 133(6), 128(3), 124(2), 118(5), 117(17), 116(8), 115(14), 105(2), 103(3), 92(14), 91(55), 90(5), 89(7), 86(12), 82(6), 81(100), 79(5), 78(5), 77(9), 70(3), 66(2), 65(27),64(3), 63(8), 62(2), 58(3), 55(4), 54(6), 53(88), 52(20), 51(26), 50(11), 44(3), 43(3), 42(14), 41(8), 40(3), 39(23), 38(4).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.31 - 7.37 (2H, m, H-13, 17), 7.24 - 7.30 (1H, m, H-15), 7.18 - 7.24 (2H, m, H-14, 16), 4.64 - 4.75 (1H, m, H-9), 4.16 - 4.27 (2H, m, H-10), 3.30 (1H, dd, *J* = 13.4 Hz, *J* = 7.8 Hz, H-11), 3.20 (2H, ddd, *J* = 7.2 Hz, *J* = 3.8 Hz, *J* = 1.5 Hz, H-2), 2.80 (1H, dd, *J* = 13.3 Hz, *J* = 9.6 Hz, H-11), 2.60 (2H, td, *J* = 7.1 Hz, *J* = 2.7 Hz, H-3), 2.00 (1H, t, *J* = 2.7 Hz, H-5).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 171.2 (C-1), 153.4 (C-7), 135.1 (C-12), 129.4 (C-13, 17), 128.9 (C-14, 16), 127.4 (C-15), 82.6 (C-4), 69.0 (C-5), 66.3 (C-10), 55.1 (C-9), 37.8 (C-11), 34.7 (C-2), 13.5 (C-3).

6.2.34 (*R*)-4-Benzyl-3-((*R*)-2-methylpent-4-ynoyl)oxazolidin-2-one (83)



To the flask containing **82** (1 g, 3.9 mmol) dissolved in anhydrous THF (42 mL) stirred at -78 °C under inert atmosphere NaHMDS (7.3 mL, 7.3 mmol, 1 M in THF) was added slowly dropwise for a period of 20 min via syringe pump and stirred for an hour. Methyl iodide (2.7 g, 19.5 mmol) was added dropwise via syringe pump and stirred for 4.5 h at -78 °C. The reaction was quenched with saturated NH_4Cl solution and allowed to warm up to the room temperature followed by extraction with ethyl acetate. The combined organic layers were washed with water and brine, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain the crude product as dark colored oil. Purification of the crude product by flash column chromatography using 20:1 to 1:1 pentane/ethyl acetate gradient elution mixture afforded the desired methylated product as brown colored solid. Recrystallization of this material from ether/hexanes (2:1) afforded **83** as the cream colored solid.

Yield: 898 mg (3.3 mmol, 85%, 99% *d.e.*).

M.pt. = 56 – 60 °C.

$[\alpha]_D^{20.6}$ = - 88.6 (*c* 6.1, chloroform).

R_f = 0.41 (pentane/ethyl acetate 2:1).

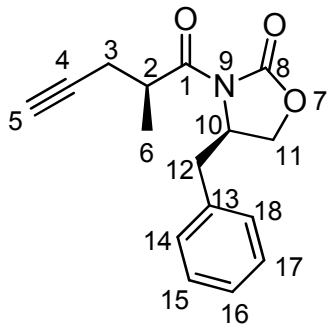
EI-MS (70 eV): *m/z* (%): 272(3) $[M+1]^+$, 271(13) $[M]^+$, 270(4), 256(2), 185(7), 181(4), 180(44), 178(3), 154(4), 137(3), 134(5), 132(6), 130(3), 128(3), 118(6), 117(30), 116(8), 114(21), 104(3), 103(5), 96(8), 95(100), 94(6), 93(3), 92(18), 91(80), 90(4), 89(9), 86(19), 79(4), 78(3), 77(10), 70(3), 68(3), 67(71), 66(19), 65(54), 64(2), 63(7), 58(4), 56(3), 55(7), 52(3), 51(11), 42(10), 41(35), 40(7), 39(28), 38(3).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.31 - 7.37 (2H, m, H-15, 17), 7.24 - 7.30 (1H, m, H-16), 7.18 - 7.23 (2H, m, H-14, 18), 4.71 (1H, ddt, *J* = 9.5 Hz, *J* = 7.2 Hz, *J* = 3.2

Hz, H-10), 4.21 (2H, dtd, *J* = 9.1 Hz, *J* = 7.2 Hz, *J* = 3.2 Hz, H-11), 3.93 (1H, dqd, *J* = 9.8 Hz, *J* = 7.0 Hz, *J* = 4.7 Hz, H-2), 3.27 (1H, dd, *J* = 13.3 Hz, *J* = 3.3 Hz, H-12), 2.78 (1H, dd, *J* = 13.3 Hz, *J* = 9.6 Hz, H-12), 2.58 (1H, ddd, *J* = 16.8 Hz, *J* = 9.8 Hz, *J* = 2.7 Hz, H-3), 2.41 (1H, ddd, *J* = 16.8 Hz, *J* = 6.8 Hz, *J* = 2.7 Hz, H-3), 1.99 (1H, t, *J* = 2.7 Hz, H-5), 1.33 (3H, d, *J* = 6.8 Hz, H-6).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 175.2 (C-1), 152.9 (C-8), 135.1 (C-13), 129.4 (C-14, 18), 128.9 (C-15, 17), 127.4 (C-16), 81.6 (C-4), 69.7 (C-5), 66.2 (C-11), 55.2 (C-10), 37.8 (C-12), 37.5 (C-2), 22.2 (C-3), 17.1 (C-6).

6.2.35 (*R*)-4-Benzyl-3-((*S*)-2-methylpent-4-ynoyl)oxazolidin-2-one (90)



To a solution of diisopropylamine (1 mL, 7.1 mmol) in freshly distilled THF (5.5 mL), n-BuLi (4.1 mL, 6.4 mmol, 1.6 M in THF) was added under insert atmosphere at 0 °C. The reaction mixture was stirred for 30 min, cooled down to -78 °C followed by the addition of HMPA (1 mL, 4.3 mmol), (*R*)-4-benzyl-3-propionyloxazolidin-2-one **65** (1 g, 4.3 mmol) in freshly distilled THF (2 mL) dropwise *via* syringe pump for 20 min at -78 °C. The resultant dark red colored solution was stirred for 30 min followed by the dropwise addition of propargylbromide **89** (1.6 mL, 17.15 mmol) at -78 °C and stirred for 20 h. The reaction mixture was quenched with saturated NH_4Cl solution and washed with water followed by the extraction with diethyl ether thrice. The combined organic extracts were washed with distilled water, dried with $MgSO_4$, filtered, and concentrated *in vacuo* to obtain the crude product as dark colored oil. Purification of the crude oil by flash chromatography using gradient elution 10:1 to 2:1 pentane/ethyl acetate mixture as eluent afforded the yellow colored powder. Recrystallization with ether/hexanes (2:1) afforded the desired product as cream colored solid. The absolute configuration of the product **90** was confirmed via X-ray structure analysis.

Yield: 911 mg (3.36 mmol, 78%, 100% *d.e.*).

M.pt. = 52-54 °C.

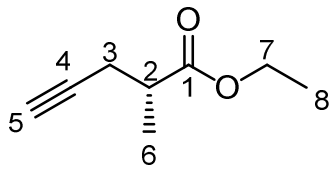
$[\alpha]_D^{20}$ = +46.4 (*c* 2.6, chloroform).

R_f = 0.45 (pentane/ethyl acetate 2:1).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ = 7.31 - 7.38 (2H, m, H-15, 17), 7.26 - 7.30 (1H, m, H-16), 7.20 - 7.26 (2H, m, H-14, 18), 4.70 (1H, ddt, *J* = 9.5 Hz, *J* = 7.1 Hz, *J* = 3.5 Hz, H-10), 4.21 (2H, dtd, *J* = 9.1 Hz, *J* = 7.2 Hz, *J* = 3.6 Hz, H-11), 3.96 (1H, sxt, *J* = 6.8 Hz, H-2), 3.30 (1H, dd, *J* = 13.4 Hz, *J* = 3.2 Hz, H-12), 2.80 (1H, dd, *J* = 13.4 Hz, *J* = 9.5 Hz, H-12), 2.62 (1H, ddd, *J* = 16.8 Hz, *J* = 9.3 Hz, *J* = 2.7 Hz, H-3), 2.50 (1H, ddd, *J* = 16.7 Hz, *J* = 6.4 Hz, *J* = 2.7 Hz, H-3), 2.03 (1H, t, *J* = 2.7 Hz, H-5), 1.29 (3H, d, *J* = 6.8 Hz, H-6).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ = 175.1 (C-1), 153.0 (C-8), 135.1 (C-13), 129.4 (C-14, 18), 128.9 (C-15, 17), 127.3 (C-16), 81.3 (C-4), 70.1 (C-5), 66.1 (C-11), 55.3 (C-10), 37.9 (C-12), 37.2 (C-2), 22.5 (C-3), 16.5 (C-6).

6.2.36 (*R*)-Ethyl 2-methylpent-4-ynoate (85)



To the solution of **83** (62 mg, 0.23 mmol) in anhydrous ethanol (2 mL) stirred under inert atmosphere Titanium(IV) ethoxide **84** (100 mg, 0.44 mmol) was added and the resultant reaction mixture was heated to reflux for 20 h. All the volatiles were carefully evaporated at 20 °C bath temperature (50 mbar) and the residue was purified by flash column chromatography using 20:1 pentane/diethyl ether mixture as eluent afforded the desired ester **85** as a fruity odor colorless liquid.[131]

Yield: 27 mg (0.19 mmol, 85%, 99% *ee*).

β-DEX chiral column, Injection mode: split ratio 20:1, Temperature program: 50 °C for 5 min, then with 5 °C/min up to 210 °C; R_t: 8.65 min.(major), 8.75 min.(minor).

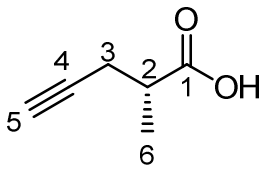
R_f = 0.78 (pentane/diethyl ether 2:1).

EI-MS (70 eV): *m/z* (%): 140(2) $[M]^+$, 138(2), 126(4), 125(47), 113(3), 112(28), 110(17), 98(7), 97(100), 96(6), 94(50), 94(2), 84(22), 83(7), 81(4), 79(8), 73(6), 70(3), 69(31), 68(14), 67(84), 66(36), 65(58), 63(8), 62(4), 56(9), 55(16), 53(11), 52(6), 51(14), 50(9), 45(11), 43(10), 42(8), 41(73), 40(14), 39(67), 38(10), 37(3).

^{1}H-NMR (600 MHz, CHLOROFORM-*d*) δ [ppm] = 4.16 (2H, qd, *J* = 7.2 Hz, *J* = 0.9 Hz, H-7), 2.65 (1H, ddq, *J* = 13.4 Hz, *J* = 6.0 Hz, *J* = 1.5 Hz, H-2), 2.53 (1H, ddd, *J* = 16.8 Hz, *J* = 6.0 Hz, *J* = 2.6 Hz, H-3), 2.37 (1H, ddd, *J* = 16.8 Hz, *J* = 7.5 Hz, *J* = 2.6 Hz, H-3), 1.99 (1H, t, *J* = 2.6 Hz, H-5), 1.32 (3H, d, *J* = 7.0 Hz, H-6), 1.27 (3H, t, *J* = 7.0 Hz, H-8).

^{13}C-NMR (151 MHz, CHLOROFORM-*d*) δ [ppm] = 174.8 (C-1), 81.5 (C-4), 69.8 (C-5), 60.6 (C-7), 38.7 (C-2), 22.6 (C-3), 16.3 (C-6), 14.2 (C-8).

6.2.37 (*R*)-2-Methylpent-4-ynoic acid (86)



To the solution of **83** (214 mg, 0.79 mmol) dissolved in 16 mL of 3:1 THF/H_2O at 0 °C under stirring 30 % aqueous H_2O_2 (0.4 mL, 4.7 mmol) and LiOH monohydrate (39 mg, 1.6 mmol) were added portionwise and allowed to stir at 0 °C for 2 h. The reaction was controlled by TLC and as soon the starting material was consumed the reaction mixture quenched by the careful and slow addition of 2 M solution of aqueous $Na_2S_2O_3$ and allowed to stir for 20 min at 0 °C. The THF was evaporated in the rotavapor and the residue was extracted with dichloromethane 4 to 5 times. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to obtain the chiral auxiliary as light colored oil. Purification of the crude chiral auxiliary by flash chromatography with 2.1 pentane/ethyl acetate mixture afforded the chiral auxiliary **64** as colorless crystals.

To the aqueous layer at 0 °C saturated solution of $NaH_2PO_4.H_2O$ was added and then acidified at 0 °C to pH 1-3 with 1N HCl solution followed by the extraction with diethyl ether five times. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to obtain **86** as light colored yellow liquid. Purification of the crude product by flash column chromatography with 1:1 pentane/diethyl ether mixture afforded the desired product **86** as colorless pungent odor liquid.

Yield: 75 mg (0.67 mmol, 85%, 96% *ee*).

β-DEX chiral column, Injection mode: split 20:1, Temperature program: 50 °C for 5 min, then with 5 °C/min up to 210 °C; R_t: 40.53 min.(major), 41.07 min.(minor).

R_f = 0.41(pentane/diethyl ether 1:1).

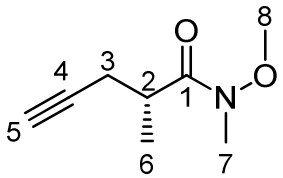
EI-MS (70 eV): *m/z* (%): 111(10) $[M-1]^+$, 98(5), 97(89), 84(8), 83(4), 79(7), 70(3), 69(40), 67(20), 66(16), 65(25), 63(6), 62(4), 56(6), 55(19), 53(10), 52(4), 51(13), 50(10), 45(37), 44(3), 43(13), 42(7), 41(80), 40(24), 39(100), 38(20), 37(10).

EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 184(2) $[M]^+$, 171(5), 170(14), 169(78), 151(2), 141(12), 130(5), 129(8), 127(6), 126(4), 125(31), 123(4), 117(12), 115(3), 112(4), 110(4), 109(3), 101(10), 99(7), 98(3), 97(7), 95(3), 93(2), 86(6), 85(8), 83(9), 79(3), 77(8), 76(12), 75(100), 74(18), 73(94), 71(2), 69(7), 67(12), 66(13), 65(16), 63(6), 62(4), 61(15), 60(5), 59(19), 58(12), 57(4), 55(11), 51(9), 50(5), 47(19), 45(46), 44(7), 43(22), 42(6), 41(31), 40(11), 39(55), 38(11), 37(3).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 11.07 (1H, br. s., -COO*H*), 2.70 (1H, qdd, *J* = 7.0 Hz, *J* = 5.9 Hz, *J* = 1.1 Hz, H-2), 2.56 (1H, ddd, *J* = 16.8 Hz, *J* = 5.9 Hz, *J* = 2.7 Hz, H-3), 2.40 (1H, ddd, *J* = 16.7 Hz, *J* = 10.2 Hz, *J* = 2.7 Hz, H-3), 2.02 (1H, t, *J* = 2.7 Hz, H-5), 1.32 (3H, d, *J* = 7.0 Hz, H-6).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 180.6 (C-1), 81.2 (C-4), 70.0 (C-5), 38.5 (C-2), 22.3 (C-3), 16.1 (C-6).

6.2.38 (*R*)-N-Methoxy-N,2-dimethylpent-4-ynamide (87)



To a stirred solution of **86** (50 mg, 0.45 mmol) in dichloromethane (2 mL) at room temperature under inert atmosphere 1,1'-carbonyldiimidazole **68** (73 mg, 0.45 mmol) was added and allowed to stir for 30 min followed by the addition of *N,O*-dimethylhyxdroxylamine hydrochloride **69** (44 mg, 0.45 mmol) in one portion. Stirring overnight for approx. 20 h, (monitored by the TLC) was needed for the consumption of the acid. The reaction mixture

was diluted with dichloromethane and the combined organic layers washed with 0.25 M HCl solution, saturated $NaHCO_3$ solution, brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to obtain crude amide **87** as fruity odor colored liquid. Purification of the crude product by flash column chromatography using gradient elution 10:1 to 3:1 pentane/diethyl ether mixture as eluent afforded the desired amide **87** as fruity odor colorless liquid.

Yield: 55 mg (0.36 mmol, 80%, 98% *ee*).

β-DEX chiral column, Injection mode: split ratio 20:1, Temperature program: 50 °C for 5 min, then with 5 °C/min up to 210 °C; R_t: 13.59 min.(major), 13.77 min.(minor).

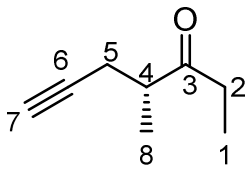
R_f = 0.22 (pentane/diethyl ether 1:1).

EI-MS (70 eV): *m/z* (%): 140(5) $[M-15]^+$, 125(11), 112(4), 97(9), 96(7), 95(63), 82(7), 68(11), 67(100), 66(16), 65(66), 63(11), 62(8), 61(31), 60(10), 58(6), 56(21), 55(16), 53(4), 52(7), 51(18), 50(12), 46(10), 45(16), 42(16), 41(93), 40(17), 39(95), 37(7), 33(8).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 3.73 (3H, s, H-8), 3.21 (3H, s, H-7), 3.08 (1H, dqd, *J* = 13.6 Hz, *J* = 6.8 Hz, *J* = 3.3 Hz, H-2), 2.52 (1H, ddd, *J* = 16.8 Hz, *J* = 9.8 Hz, *J* = 2.8 Hz, H-3), 2.28 (1H, ddd, *J* = 16.6 Hz, *J* = 7.5 Hz, *J* = 2.8 Hz, H-3), 1.99 (1H, t, *J* = 2.6 Hz, H-5), 1.22 (3H, d, *J* = 6.8 Hz, H-6).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 175.8 (C-1), 82.5 (C-4), 69.3 (C-5), 61.5 (C-8), 35.2 (C-2), 32.1 (C-7), 22.5 (C-3), 16.9 (C-6).

6.2.39 (*R*)-4-Methylhept-6-yn-3-one (88)



To a stirred solution of **87** (25 mg, 0.16 mmol) in anhydrous THF (1 mL) at -20 °C under inert atmosphere ethyl magnesium bromide **71** (0.2 mL, 0.38 mmol, 3 M in diethyl ether) was added dropwise at -20 °C. Stirring was continued until the consumption of the amide was detected by TLC. The reaction mixture was quenched by slow addition of saturated NH_4Cl solution and extracted with three to five times with diethyl ether. The combined organic

layers were dried with $MgSO_4$, filtered, and concentrated in rotavapor to obtain the crude chiral allyl ketone. Purification of the crude product by flash chromatography using 5:1 pentane/diethyl ether mixture as eluent afforded chiral allyl ketone **88** as fruity odor colorless volatile liquid.

Yield: 14 mg (0.11 mmol, 70%, 98% *ee*).

β-DEX chiral column, Injection mode: split ratio 20:1, Temperature program: 50 °C for 1 min, then with 5 °C/min up to 210 °C; R_t: 9.62 min.(major), 9.79 min.(minor).

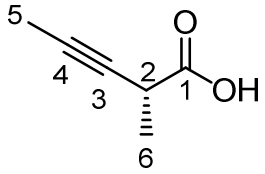
R_f = 0.55 (pentane/diethyl ether 1:1).

EI-MS (70 eV): *m/z* (%): 124(5) $[M]^+$, 122(8), 109(30), 96(7), 95(66), 68(4), 67(61), 66(10), 65(33), 63(6), 58(4), 57(100), 55(7), 53(5), 52(5), 51(11), 50(8), 43(4), 42(5), 41(55), 40(10), 39(57), 38(11), 37(4).

^{1}H-NMR (600 MHz, CHLOROFORM-*d*) δ [ppm] = 2.67 (1H, sxt, *J* = 7.2 Hz, H-4), 2.47 (2H, ddq, *J* = 21.6 Hz, *J* = 17.7 Hz, *J* = 7.2 Hz, H-2), 2.40 (1H, ddd, *J* = 16.9 Hz, *J* = 7.3 Hz, *J* = 2.6 Hz, H-5), 2.21 (1H, ddd, *J* = 16.9 Hz, *J* = 7.3 Hz, *J* = 2.6 Hz, H-5), 1.90 (1H, t, *J* = 2.6 Hz, H-7), 1.12 - 1.14 (4H, m, H-8), 0.99 (3H, t, *J* = 7.3 Hz, H-1).

^{13}C-NMR (151 MHz, CHLOROFORM-*d*) δ [ppm] = 212.9 (C-3), 82.0 (C-6), 69.6 (C-7), 44.9 (C-4), 34.1 (C-2), 22.3 (C-5), 16.3 (C-8), 7.6 (C-1).

6.2.40 (*R*)-2-Methylpent-3-ynoic acid (78)



KO*t*Bu (0.4 g, 3.6 mmol), anhydrous DMSO (2 mL) were added to acid with terminal alkyne **86** (200 mg, 1.8 mmol) under inert atmosphere at room temperature. The resulting solution changed from colorless to orange and stirred for 4 h monitored by GC/MS for completion of the reaction. The reaction mixture quenched with 2 M HCl solution to pH 2-3 and extracted five times with diethyl ether. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude product by flash column

chromatography with 2:1 pentane/diethyl ether mixture as eluent afforded the internal alkyne **78** as light yellow colored liquid.[96]

Yield: 108 mg (0.9 mmol, 54%).

R_f = 0.11 (pentane/diethyl ether 2:1).

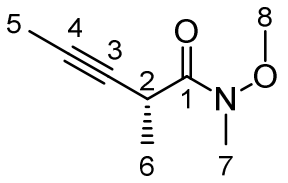
EI-MS (70 eV): *m/z* (%):112(26) $[M]^+$, 111(15), 97(109, 95(3), 94(4), 84(6), 83(7), 69(16), 67(27), 66(32), 65(32), 64(4), 62(15), 61(7), 57(2), 56(6), 55(10), 53(13), 52(14), 51(21), 50(17), 49(4), 45(86), 43(6), 41(39), 40(17), 39(100), 38(32), 37(16).

EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 185(4) $[M+1]^+$, 184(19) $[M]^+$, 171(5), 170(14), 169(100), 141(8), 125(12), 111(4), 95(39), 94(7), 85(7), 75(31), 73(30), 67(30), 66(17), 65(19), 63(4), 59(8), 58(6), 51(3).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 10.48 (1H, br. s., -COO*H*), 3.43 (1H, q, *J* = 6.9 Hz, H-2), 1.93 (3H, t, *J* = 2.7 Hz, H-5), 1.25 (3H, d, *J* = 7.0 Hz, H-6).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 177.9 (C-1), 82.1 (C-4), 69.2 (C-3), 33.2 (C-2), 14.1 (C-6), 1.0 (C-5).

6.2.41 (*R*)-*N*-Methoxy-*N*,2-dimethylpent-3-ynamide (79)



To a stirred solution of **78** (50 mg, 0.45 mmol) in dichloromethane (2 mL) at room temperature under inert atmosphere 1,1'-carbonyldiimidazole **68** (73 mg, 0.45 mmol) was added and allowed to stir for 30 min followed by the addition of *N,O*-dimethylhyxdroxylamine hydrochloride **69** (44 mg, 0.45 mmol) in one portion. Stirring overnight for approx. 20 h, (monitored by the TLC) was need for the consumption of the acid. The reaction mixture was diluted with dichloromethane and the combined organic layers washed with 0.25 M HCl solution, saturated $NaHCO_3$ solution and brine. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to obtain crude amide **79** as fruity odor light yellow colored liquid. Purification of the crude product by flash column

chromatography using gradient elution 10:1 to 3:1 pentane/diethyl ether mixture as eluent afforded the desired amide **79** as fruity odor colorless liquid.

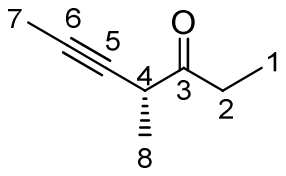
Yield: 16 mg (0.1 mmol, 78%, 99% *ee*).

β-DEX chiral column, Injection mode: split ratio 20:1, Temperature program: 50 °C for 5 min, then with 10 °C/min up to 210 °C; R_t: 13.41 min.(major), 13.59 min.(minor).

R_f = 0.21 (pentane/ethyl acetate 1:1).

EI-MS (70 eV): *m/z* (%): 155(6) $[M]^+$, 125(3), 124(2), 96(6), 95(100), 68(4), 67(70), 66(10), 65(26), 63(4), 56(3), 51(4), 41(34), 39(23).

6.2.42 (*R*)-4-Methylhept-5-yn-3-one (80)



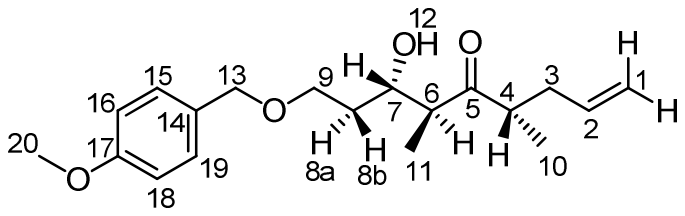
To a stirred solution of **79** (10 mg, 0.06 mmol) in anhydrous THF (1 mL) at 0 °C under inert atmosphere ethyl magnesium bromide **71** (0.05 mL, 0.2 mmol, 3 M in diethyl ether) was added dropwise at -20 °C. Stirring was continued until the consumption of the amide was detected by TLC. The reaction mixture was quenched by slow addition of saturated NH_4Cl solution and extracted three to five times with diethyl ether. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated in rotavapor to obtain the crude chiral allyl ketone. Purification of the crude product by flash chromatography using 5:1 pentane/diethyl ether mixture as eluent afforded chiral allyl ketone **80** as fruity odor light yellow volatile liquid with minor impurities as analyzed by GC/MS.

Yield: ~ 5 mg (0.03 mmol, 50%).

R_f = 0.55 (pentane/diethyl ether 1:1).

EI-MS (70 eV): *m/z* (%): 124(44) $[M]^+$, 109(4), 96(5), 95(96), 91(7), 85(8), 84(8), 81(13), 79(8), 69(10), 67(6), 67(100), 66(16), 65(34), 57(6), 53(11), 51(7).

6.2.43 (4*S*,6*S*,7*S*)-7-Hydroxy-9-((4-methoxybenzyl)oxy)-4,6-dimethylnon-1-en-5-one (91)



To a stirred solution of dicyclohexylborane (0.5 mL, 0.5 mmol, 1M in hexane) in dry diethylether (1 mL) under inert atmosphere at 0 °C, triethylamine (62 mg, 0.61 mmol) was added dropwise and stirred for 1 h. To this reaction mixture (*S*)-chiral ketone **61** (52 mg, 0.41 mmol) in dry diethyl ether (1 mL) was added dropwise and stirred at 0 °C for 4 h. The reaction mixture cooled down to -78 °C, aldehyde **41** (110 mg, 0.57 mmol) in dry diethylether (1 mL) was added dropwise and stirred for 2 h. The reaction mixture was left overnight (16 h) in a freezer at -32 °C approx. temperature.[97]

The reaction mixture was quenched at 0 °C with pH 7 buffer (4 mL) and extracted with diethylether (3 x 20 mL). The combined organic layers were concentrated in *vacuo*. The crude oil was suspended in methanol (5 mL) and pH 7 buffer (1 mL) followed by addition of hydrogen peroxide (1.5 mL, 30% aq.) dropwise at 0 °C. The reaction mixture was stirred for 2 h, poured into water (8 mL) and extracted with dichloromethane (3 x 20 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution and brine, dried with $MgSO_4$, filtered, and concentrated in *vacuo*. The crude product was purified by flash column chromatography with 10:1 diethyl ether/dichloromethane mixture as eluent to afford aldol product **91** as colorless oil.[63]

Yield: 104 mg (0.32 mmol, 80%).

R_f = 0.64 (diethyl ether/dichloromethane 10:1); 0.22 (pentane/ethyl acetate 5:1);

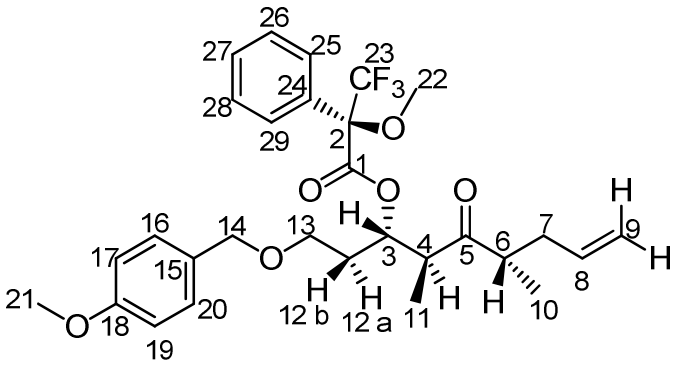
R_f = 0.12 (pentane/diethyl ether 2:1).

EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 392(1) $[M]^+$, 318(1), 267(2), 227(2), 211(2), 209(8), 202(2), 183(2), 177(7), 176(42), 166(5), 157(7), 145(3), 137(25), 135(8), 131(9), 122(25), 121(100), 119(2), 115(5), 109(4), 101(4), 97(22), 91(8), 78(9), 77(12), 75(16), 73(23), 69(28), 67(3), 59(3), 55(3), 45(3), 41(13), 39(2).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.22 - 7.27 (2H, m, H-15, 19), 6.85 - 6.90 (2H, m, H-16, 18), 5.71 (1H, dddd, $J_{2,1}$ = 16.9 Hz, $J_{2,1}$ = 10.1 Hz, $J_{2,3}$ = 7.6 Hz, $J_{2,3}$ = 6.6 Hz, H-2), 5.02 (2H, m, J_E = 16.9 Hz, J_Z = 9.1 Hz, ^{2}J = 1.5 Hz, H-1), 4.44 (2H, s, H-13), 3.86 - 3.95 (1H, m, $J_{7,6}$ = 9.6 Hz, *J* = 7.1 Hz, $J_{7,8b}$ = 2.8 Hz, H-7), 3.80 (3H, s, H-20), 3.66 - 3.72 (1H, m, *J* = 9.1, *J* = 7.1, *J* = 4.8, H-9), 3.58 - 3.65 (1H, m, *J* = 9.1, *J* = 7.1, *J* = 4.8, H-9), 3.26 (1H, d, $^{3}J_{12,7}$ = 4.5 Hz, H-12 major *ds*), 3.28 (1H, d, $^{3}J_{12,7}$ = 4.5 Hz, H-12 minor *ds*), 2.79 (1H, m, $J_{6,11}$ = 7.1 Hz, H-6), 2.71 (1H, qd, $J_{4,3}$ = 7.6 Hz, $J_{4,10}$ = 6.8 Hz, H-4), 2.42 (1H, tsxt, *J* = 13.9 Hz, $J_{3,4}$ = 7.6 Hz, *J* = 6.3 Hz, ^{4}J = 1.52, H-3), 2.03 (1H, tsxt, *J* = 13.9 Hz, $J_{3,4}$ = 7.6 Hz, *J* = 6.3 Hz, ^{4}J = 1.26, H-3), 1.76 - 1.79 (1H, m, H-8b major *ds*), 1.80-1.83 (1H, m, H-8b minor *ds*), 1.65 - 1.74 (1H, m, $J_{8a,7}$ = 2.8 Hz, H-8a), 1.08 (3H, d, $J_{11,6}$ = 7.1 Hz, H-11), 1.06 (3H, d, $J_{10,4}$ = 6.8 Hz, H-10).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 218.1 (C-5), 159.2 (C-17), 135.8 (C-2), 130.0 (C-14), 129.4 (C-15, 19), 116.8 (C-1), 113.8 (C-16,18), 73.0 (C-13), 72.9 (C-7), 68.3 (C-9), 55.3 (C-20), 50.4 (C-6), 45.9 (C-4), 36.6 (C-3), 33.8 (C-8), 15.6 (C-11), 13.7 (C-10).

6.2.44 (*S*)-(3*S*,4*S*,6*S*)-1-((4-Methoxybenzyl)oxy)-4,6-dimethyl-5-oxonon-8-en-3-yl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (97)



To a stirred solution of aldol product **91** (25 mg, 0.078 mmol) in dichloromethane (1 mL) under inert atmosphere at room temperature DMAP (5.24 mg, 0.043 mmol), triethylamine (39 mg, 0.39 mmol) and (*R*)-MTPACl **95** (37 µL, 0.20 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. Stirring was continued until the consumption of the starting material was detected by TLC. The reaction mixture was purified by directly loading on the flash column chromatography using 50:1 to 10:1 gradient elution with pentane/diethyl ether mixture as eluent to afford **97** as colorless oil.[99]

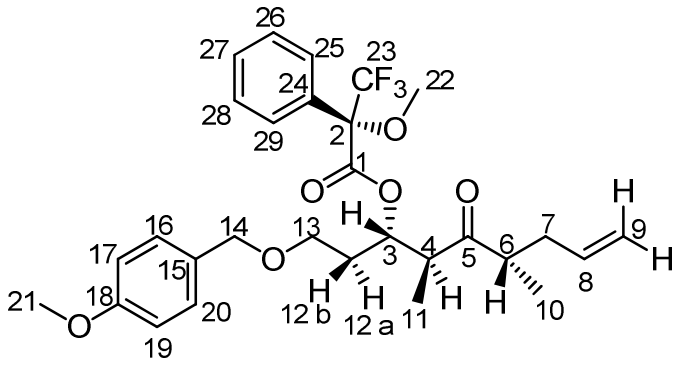
Yield: 21 mg (0.039 mmol, 50%).

R_f = 0.31 (pentane/diethyl ether 2:1).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.46 – 7.50 (2H, m, H-25, 29), 7.36 – 7.40 (2H, m, H-26, 28), 7.35 – 7.39 (1H, m, H-27), 7.19 – 7.24 (2H, m, H-16, 20), 6.97 – 6.98 (CF_3), 6.83 - 6.88 (2H, m, H-17, 19), 5.56 – 5.63 (1H, m, H-8), 5.46 – 5.50 (1H, m, H-3), 4.97 – 5.00 (2H, m, H-9 *major isomer*), 4.93 – 4.96 (2H, m, H-9 *minor isomer*), 4.38 (2H, s, H-14), 3.79 (3H, s, H-21), 3.45 (3H, s, H-22), 3.38 – 3.46 (2H, m, H-13), 3.09 – 3.15 (1H, m, H-4), 2.62 – 2.68 (1H, m, H-6), 2.20 – 2.28 (1H, m, H-7), 1.78 – 1.98 (2H, m, H-12), 1.75 – 1.84 (1H, m, H-7), 1.01 – 1.03 (3H, d, H-10), 0.96 – 0.98 (3H, d, H-11).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 213.1 (C-5), 166.0 (C-1), 159.2 (C-18), 135.7 (C-8), 132.1 (C-24), 130.2 (C-15), 129.6 (C-27), 129.2 (C-16, 20), 128.4 (C-26, 28), 127.5 (C-23), 127.3 (C-25, 29), 125.5 (C-23), 124.7 (C-23), 121.8 (C-23), 117.0 (C-9), 113.8 (C-17, 19), 84.7 (C-2), 84.4 (C-2), 74.3 (C-3), 72.6 (C-14), 65.5 (C-13), 55.4 (C-22), 55.2 (C-21), 47.2 (C-4), 45.0 (C-6), 36.4 (C-7), 30.0 (C-12), 15.9 (C-10), 11.2 (C-11).

6.2.45 (*R*)-(3*S*,4*S*,6*S*)-1-((4-Methoxybenzyl)oxy)-4,6-dimethyl-5-oxonon-8-en-3-yl 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (98)



To a stirred solution of aldol product **91** (25 mg, 0.078 mmol) in dichloromethane (1 mL) under inert atmosphere at room temperature DMAP (5.24 mg, 0.043 mmol), triethylamine (39 mg, 0.39 mmol) and (*S*)-MTPACl **96** (37 µL, 0.20 mmol) were added. The reaction mixture was stirred for 16 h at room temperature. Stirring was continued until the consumption of the starting material was detected by TLC. The reaction mixture was purified by directly loading on the flash column chromatography using 50:1 to 5:1 gradient elution with pentane/diethyl ether mixture as eluent to afford **98** as colorless oil.[99]

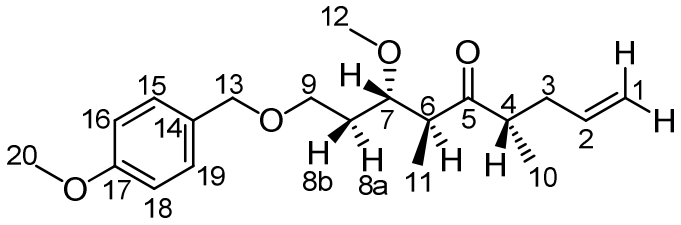
Yield: 37 mg (0.069 mmol, 88%).

R_f = 0.29 (pentane/diethyl ether 2:1).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.45 – 7.49 (2H, m, H-25, 29), 7.35 – 7.40 (3H, m, H-26, 27, 28), 7.19 – 7.23 (2H, m, H-16, 20), 6.98 – 6.99 (s, CF_3), 6.83 – 6.87 (2H, m, H-17, 19), 5.56 – 5.66 (1H, m, H-8), 5.49 – 5.51 (1H, m, H-3 minor *ds*), 5.46 – 5.48 (1H, m, H-3 major *ds*), 5.02 – 5.04 (2H, m, H-9 *minor isomer*), 4.93 – 5.01 (2H, m, H-9 *major isomer*), 4.38 (2H, s, H-14), 3.79 (3H, s, H-21), 3.47 (3H, s, H-22), 3.30 – 3.35 (1H, m, H-13), 3.20 – 3.27 (1H, m ,H-13), 3.06 – 3.08 (1H, m, H-4 minor *ds*) , 3.15 – 3.18 (1H, m, H-4 major *ds*) , 2.64 – 2.71 (1H, m, H- 6), 2.29 -2.32 (1H, m, H-7 minor *ds*), 2.21 – 2.32 (1H, m, H-7 major *ds*), 1.96 – 2.00 (1H, m, H-7 minor *ds*), 1.82 – 1.87 (1H, m, H-7 major *ds*), 1.78 – 1.90 (2H, m, H-12), 1.07 – 1.08 (3H, d, H-11), 1.04 – 1.06 (3H, d, H-10).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 213.3 (C-5), 166.1 (C-1), 159.1 (C-18), 135.6 (C-8), 132.0 (C-24), 130.3 (C-15), 129.6 (C-27), 129.1 (C-16, 20), 128.4 (C-26, 28), 127.3 (C-25, 29), 125.5 (C-23), 124.8 (C-23), 121.9 (C-23), 117.0 (C-9), 113.8 (C-17, 19), 84.7 (C-2), 84.4 (C-2), 74.5 (C-3), 72.6 (C-14), 65.2 (C-13), 55.4 (C-22), 55.2 (C-21), 47.2 (C-4), 45.1 (C-6), 36.5 (C-7), 29.8 (C-12), 15.9 (C-10), 11.6 (C-11).

6.2.46 (4*S*,6*S*,7*S*)-7-Methoxy-9-((4-methoxybenzyl)oxy)-4,6-dimethylnon-1-en-5-one (99)



To a stirred solution of proton sponge® (257 mg, 1.2 mmol) and trimethyloxonium tetrafluoroborate (177 mg, 1.2 mmol) in dichloromethane (12 mL), a solution of aldol **91** (48 mg, 0.15 mmol) in dichloromethane (2 mL) was added at room temperature. The reaction mixture was stirred for 18 h and saturated aqueous $NaHCO_3$ was added. Diethyl ether was added until the organic layer stayed on top. Layers were separated and the aqueous layer was extracted with diethyl ether (3 x 20 mL).The combined organic layers were washed several times with an aqueous $KHSO_4$ solution (1.5 M) until the color of the organic layer faded from brown to nearly colorless. Then the organic layer was washed with brine, dried with $MgSO_4$, filtered, and concentrated in *vacuo*. Purification by flash column

chromatography with 5:1 pentane/ethyl acetate mixture as eluent provided methylated product **99** as colorless oil.[102,103]

Yield: 40 mg (0.12 mmol, 80%).

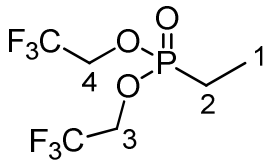
R_f = 0.56 (pentane/ethyl acetate 5:1).

EI-MS (70 eV): *m/z* (%): 334(1) $[M]^+$, 293(2), 181(1), 176(13), 166(2), 137(11), 136(4), 135(7), 125(3), 122(13), 121(100), 109(3), 107(2), 97(11), 91(6), 89(3), 78(10), 77(11), 72(3), 69(24), 67(4), 65(3), 63(2), 58(4), 57(2), 55(4), 52(3), 51(2), 43(3), 41(24), 39(6).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.23 - 7.28 (2H, m, H-15, 19), 6.86 - 6.90 (2H, m, H-16, 18), 5.72 (1H, dddd, *J* = 13.6 Hz, 10.3 Hz, 6.8 Hz, 3.5 Hz, H-2), 4.97 - 5.07 (2H, m, H-1), 4.43 (2H, s, H-13), 3.80 (3H, s, H-20), 3.57 - 3.60 (1H, m, H-7), 3.51 - 3.56 (2H, m, H-9), 3.25 (3H, s, H-12), 2.91 (1H, qd, *J* = 7.0 Hz, 1.3 Hz, H-6), 2.69 (1H, qdd, *J* = 6.8 Hz, 3.0 Hz, 1.3 Hz, H-4), 2.34 - 2.49 (1H, m, H-3), 1.97 - 2.09 (1H, m, H-3), 1.89 (1H, dtt, *J* = 14.4 Hz, 7.1 Hz, 3.4 Hz, H-8a), 1.58 - 1.69 (1H, m, H-8b), 1.07 (3H, d, *J* = 7.0 Hz, H-10), 0.97 (3H, d, *J* = 7.0 Hz, H-11).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 216.2 (C-5), 159.1 (C-17), 136.1 (C-2), 130.5 (C-14), 129.1 (C-15, 19), 116.5 (C-1), 113.7 (C-16, 18), 80.2 (C-7), 72.6 (C-13), 65.9 (C-9), 58.2 (C-12), 55.2 (C-20), 48.3 (C-6), 46.3 (C-4), 36.2 (C-3), 31.1 (C-8), 15.7 (C-10), 12.9 (C-11).

6.2.47 bis(2,2,2-Trifluoroethyl) ethylphosphonate (105)



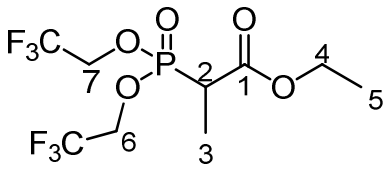
To a stirred solution of trifluoroethanol **104** (5 mL, 66 mmol) and Et_3N (11 mL, 79 mmol) in anhydrous THF (96 mL) at 0 °C under inert atmosphere, ethyl phosphonicdichloride **103** (8.7 g, 59 mmmol) dissolved in anhydrous THF (15 mL) was added dropwise via a dropping funnel. The reaction mixture stirred vigorously at room temperature for 2 h 20 min and filtered. The filtered cake was washed with THF and concentrated in vacuo to obtain the crude product. Vacuum distillation of the crude product over oil bath at 50-160 °C (53 mbar) afforded the product **105** as a colorless oil.[107]

yield: 5 g (18.24 mmol, 31%).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 4.11 - 4.24 (4H, m, *J* = 12.5 Hz, *J* = 7.6 Hz, *J* = 7.6 Hz, *J* = 7.6 Hz, J = 7.6 Hz, H-3, 4), 1.86 (2H, dq, *J* = 17.6 Hz, *J* = 6.8 Hz, H-2), 1.33 (3H, dt, *J* = 19.1 Hz, *J* = 7.0 Hz, H-1).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] =121.9(2 x CF_3), 60.0 (C-3, 4), 20.6 (C-2), 14.2 (C-1).

6.2.48 Ethyl 2-(bis(2,2,2-trifluoroethoxy)phosphoryl)propanoate (109)



To the solution of LiHMDS (41 mL, 40.46 mmol, 1M in hexane) in freshly distilled THF(40 mL) at -78 °C under inert atmosphere, triflouroethyl-ethyl phosphonate **105** (5 g, 18.24 mmol) was added and stirred for 10 min. To the reaction mixture ethyl-chloroformate **106** (2 mL, 20 mmol) was slowly added dropwise and stirred for 1 h and kept in a freezer (-20 °C) for overnight. The reaction mixture poured into 2 M HCl, equal amounts of crushed ice and dichloro methane. The organic layer was separated and aqueous layer was extracted with dichloro methane and the combined organic extracts were washed with water, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product obtained was purified by flash column chromatography with 3:1 pentane/ethyl acetate mixture as eluent to afford the desired phosphonate ester **109** as colorless oil in 30% yield.[107]

To the solution of NaH (60 % dispersed in oil, 1.6 g, 40.4 mmol) in freshly distilled THF (6 mL) at room temperature under inert atmosphere HMPA (6.1 mL, 35 mmol) was added. To this solution bis(2,2,2-Trifluoroethyl) phosphite **107** (5 g, 20.32 mmol) was aadded dropwise and stirred for 10 min. To the reaction mixture ethyl-2-bromo propionate **108** (2 mL, 15.84 mmol) was slowly added and stirred for 5 h at room temperature. The reaction quenched with saturated NH_4Cl solution extracted with TBME and the combined organic extracts were washed with water, brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product obtained was purified by flash column chromatography with 3:1 pentane/ethyl acetate mixture as eluent afforded the desired phosphonate ester **109** as colorless oil.[105]

Yield: 2.4 g (7 mmol, 35%).

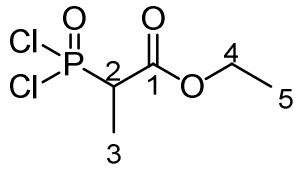
R_f = 0.74 (pentane/ethyl acetate 3:1).

EI-MS (70 eV): *m/z* (%): 347(8) $[M+1]^+$, 346(20) $[M]^+$, 319(36), 302(17), 301(99), 300(9), 280(27), 275(31), 274(100), 273(85), 271(3), 254(36), 253(11), 247(19), 246(99), 245(40) 243(44), 226(22), 225(25), 219(11), 203(4), 193(6), 191(8), 176(9), 175(15), 174(5), 173(13), 165(13), 163(50), 147(9), 143(16), 127(9), 115(13), 113(8), 101(7), 99(18), 93(12), 91(17), 83(21), 81(10), 80(5), 73(8), 69(15), 67(7), 64(22), 61(13), 56(18), 55(14), 47(9), 45(8), 43(6), 33(13).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 4.32 - 4.52 (4H, m, H-6, 7), 4.22 (2H, q, *J* = 7.2 Hz, H-4), 3.19 (1H, dq, *J* = 22.5 Hz, *J* = 7.4 Hz, H-2), 1.50 (3H, dd, *J* = 19.3 Hz, *J* = 7.4 Hz, H-3), 1.30 (3H, t, *J* = 7.2 Hz, H-5).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 170.2 (C-1), 122.0(2xCF_3), 62.2 (C-6, 7), 61.2 (C-4), 39.5 (C-2), 14.1 (C-5), 11.5 (C-3).

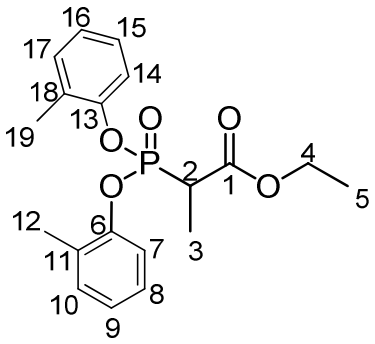
6.2.49 Ethyl 2-(dichlorophosphoryl)propanoate (112)



To the solution of ethyl 2-(diethoxyphosphoryl)propanoate **110** (4.8 g, 18.9 mmol) in anhydrous benzene (35 mL) stirred at 0 °C (ice bath) under inert atmosphere, PCl_5 **111** (12 g, 78 mmol) was added slowly (exothermic reaction). After the addition the reaction mixture was heated to 75 °C for 10 h. The reaction mixture was carried for the next step without further purification.[109]

EI-MS (70 eV): *m/z* (%): 218(2) $[M+1]^+$, 217(3) $[M]^+$, 194(2), 192(11), 190(5), 190(18), 189(5), 173(2), 166(10), 165(6), 164(62), 163(25), 162(100), 161(32), 154(6), 153(4), 147(3), 145(8), 143(5), 129(6), 128(7), 127(20), 126(18), 125(11), 119(4), 117(6), 111(6), 110(3), 109(6), 108(8), 107(6), 101(10), 100(6), 99(28), 97(2), 92(12), 91(13), 90(34), 89(7), 85(6), 83(17), 81(5), 79(5), 77(2), 73(2), 65(12), 64(9), 63(9), 62(26), 61(10), 55(9), 54(6), 53(5), 48(3), 47(25), 45(11), 43(63), 42(6), 41(2).

6.2.50 Ethyl 2-(bis(*o*-tolyloxy)phosphoryl)propanoate (114)



Dichloro phosphoryl propaonate **112** was cooled down to 0 °C under inert atmosphere Et_3N (4.4 g, 43.6 mmol), *o*-cresol **113** (4.7 g, 43.65 mmol) in anhydrous benzene (12 mL) was added and stirred for 1 h at room temperature. The reaction mixture was filtered and the filtrate was extracted with ethyl acetate, washed with 1 M NaOH solution, saturated NH_4Cl solution, dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography with 8:1 hexane/ethyl acetate mixture afforded the desired di-tolyl phosphonate **114** as colorless oil.[109]

Yield: 5.1 g (14.2 mmol, 75% over two steps).

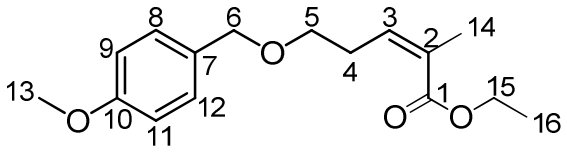
R_f = 0.23 (pentane/TBME 1:1).

EI-MS (70 eV): *m/z* (%): 363(13) $[M+1]^+$, 362(59) $[M]^+$, 317(25), 316(8), 315(6), 289(6), 288(8), 274(7), 273(39), 255(10), 228(11), 227(100), 209(14), 199(6), 198(7), 197(8), 183(11), 182(7), 181(11), 180(10), 179(17), 178(10), 171(10), 165(8), 163(7), 153(7), 135(10), 119(7), 117(6), 109(7), 108(23), 107(31), 105(7), 92(6), 91(65), 90(22), 89(18), 79(15), 78(17), 77(38), 65(30), 63(6), 51(8), 39(7).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.20 - 7.32 (4H, m, H-8, 10, 15, 17), 7.15 (1H, tt, *J* = 7.3 Hz, *J* = 2.0 Hz, H-7), 7.09 (1H, tt, *J* = 7.8 Hz, *J* = 1.5 Hz, H-14), 7.04 (2H, t, *J* = 7.3 Hz, H-9, 16), 4.21 (2H, q, *J* = 7.0 Hz, H-4), 3.44 (1H, dq, *J* = 23.8 Hz, *J* = 7.3 Hz, H-2), 2.19 (6H, d, *J* = 14.1 Hz, H-12, 19), 1.68 (3H, dd, *J* = 19.1 Hz, *J* = 7.3 Hz, H-3), 1.23 (3H, t, *J* = 7.2 Hz, H-5).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 168.7 (C-1), 148.9 (C-6, 13), 131.3 (C-10, 17), 129.2 (C-11, 18), 126.9 (C-8, 15), 125.0 (C-9, 16), 120.1 (C-7, 14), 61.7 (C-4), 39.9 (C-2), 16.2 (C-12, 19), 13.9 (C-5), 11.8 (C-3).

6.2.51 (*Z*)-Ethyl 5-((4-methoxybenzyl)oxy)-2-methylpent-2-enoate (115)



To a solution of phosphonoate reagent (2.02 g, 5.6 mmol) in freshly distilled THF (14 mL) stirred under inert atmosphere at 0 °C, NaI (1 g, 6.75 mmol) was added, followed by dropwise addition of DBU (822 mg, 5.4 mmol) and stirred for 10 minutes. The reaction mixture cooled down to -78 °C and aldehyde (1.1 g, 5.6 mmol) in freshly distilled THF (8 mL) was added slowly and stirred for 2 h at -78 °C. It was stirred further for 2h at room temperature. The reaction mixture quenched with saturated NH_4Cl solution, extracted thrice with TBME. The combined organic extracts were washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 2:1 pentane/TBME mixture as eluent to afford the desired (*Z*)-configured ester **115** exclusively as a fruity odor colorless liquid.[108]

Yield: 1.31 g (4.7 mmol, 84%).

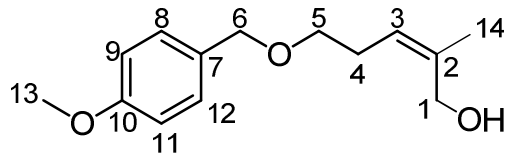
R_f = 0.28 (pentane/TBME 1:1).

EI-MS (70 eV): *m/z* (%): 278(1) $[M]^+$, 260(1), 248(2), 234(1), 233(1), 232(2), 206(1), 205(8), 204(1), 201(2), 187(1), 186(1), 161(2), 159(2), 142(5), 138(2), 137(4), 136(3), 135(5), 122(9), 121(100), 114(3), 113(2), 111(2), 109(2), 107(2), 106(3), 98(3), 97(2), 94(2), 92(2), 91(7), 90(2), 89(4), 84(2), 83(2), 82(3), 79(2), 78(17), 77(15), 71(1), 69(2), 67(4), 65(4), 63(3), 55(5), 54(4), 53(6), 52(4), 51(4), 50(2), 45(1), 43(6), 41(5), 39(7).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.15 - 7.21 (2H, m, *J* = 8.7 Hz, H-8, 12), 6.76 - 6.82 (2H, m, *J* = 8.7 Hz, *J* = 2.7 Hz, H-9, 11), 5.93 (1H, tq, *J* = 7.1 Hz, *J* = 1.4 Hz, H-3), 4.37 (2H, s, H-6), 4.10 (2H, q, *J* = 7.2 Hz, H-15), 3.71 (3H, s, H-13), 3.43 (2H, t, *J* = 6.4 Hz, 5), 2.69 (2H, dt, *J* = 7.8 Hz, *J* = 6.4 Hz, H-4), 1.83 (3H, q, *J* = 1.3 Hz, H-14), 1.21 (3H, t, *J* = 7.2 Hz, H-16).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 167.8 (C-1), 159.1 (C-10), 139.3 (C-3), 130.4 (C-7), 129.2 (C-8, 12), 128.6 (C-2), 113.7 (C-9, 11), 72.4 (C-6), 69.1 (C-5), 60.0 (C-15), 55.2 (C-13), 30.1 (C-4), 20.6 (C-14), 14.2 (C-16).

6.2.52 (*Z*)-5-((4-Methoxybenzyl)oxy)-2-methylpent-2-en-1-ol (116)



To the solution of ester **115** (876 mg, 3.15 mmol) in anhydrous diethyl ether (6 mL) under inert atmosphere at 0 °C, LAH (280 mg, 7.4 mmol) was added portion wise and stirred for 15 minutes. The reaction quenched up with saturated Na_2SO_4 solution at 0 °C, stirred for 15 minutes and the precipitated solid was filtered through a pad of celite, washed with diethyl ether. The organic extract was washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude product with flash column chromatography using 3:1 pentane/TBME as eluent afforded (*Z*)-allyl alcohol **116** as a colorless oil.

Yield: 728 mg (3.1 mmol, 98%).

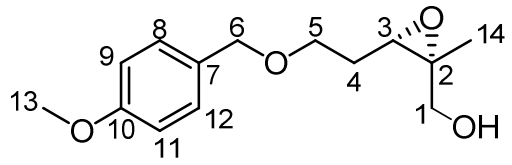
R_f = 0.24 (pentane/TBME 1:1).

EI-MS (70 eV): *m/z* (%): 236(1) $[M]^+$, 218(1), 205(1), 152(4), 151(6), 149(1), 147(1), 138(4), 137(20), 136(22), 135(14), 123(3), 122(36), 121(100), 119(1), 115(2), 109(4), 108(2), 107(6), 106(4), 104(2), 94(3), 92(4), 91(12), 90(4), 89(5), 85(2), 83(2), 79(4), 78(24), 77(22), 67(4), 65(5), 63(3), 57(3), 55(4), 53(4), 51(4), 43(9), 41(8), 39(6).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.20 - 7.28 (2H, m, *J* = 8.5 Hz, H-8, 12), 6.84 - 6.91 (2H, m, *J* = 8.9 Hz, *J* = 2.3 Hz, H-9, 11), 5.31 (1H, td, *J* = 7.9 Hz, *J* = 1.5 Hz, H-3), 4.43 (2H, s, H-6), 4.01 (2H, d, *J* = 0.4 Hz, H-1), 3.79 (3H, s, H-13), 3.43 (2H, t, *J* = 6.1 Hz, H-5), 2.25 - 2.39 (2H, m, *J* = 7.8 Hz, *J* = 6.8 Hz, H-4), 1.81 (3H, ddd, *J* = 3.6 Hz, *J* = 1.5 Hz, *J* = 0.6 Hz, H-14).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 159.2 (C-10), 137.8 (C-2), 129.7 (C-7), 129.4 (C-8, 12), 124.5 (C-3), 113.7 (C-9, 11), 72.7 (C-6), 68.8 (C-5), 61.0 (C-1), 55.1 (C-13), 28.3 (C-4), 22.1 (C-14).

6.2.53 ((2*R*,3*S*)-3-(2-((4-Methoxybenzyl)oxy)ethyl)-2-methyloxiran-2-yl)methanol (117)



To a suspension of activated 4 A° molecular sieves (55 mg, 20 wt %) in anhydrous DCM (5 mL) at -30 °C under inert atmosphere Ti(O*i*-Pr)$_4$(330 mg, 1.2 mmol), (-)-DET (0.4 mL, 1.4 mmol) was added and stirred for 30 minutes. Allylic alcohol **116** (273 mg, 1.2 mmol) in anhydrous DCM (4 mL) was added dropwise using a syringe pump over a period of 25 minutes at -35 °C. The reaction mixture stirred for 30 minutes followed by the addition of TBHP (1.4 mL, 7.5 mmol, 5.5 M in nonane) at -35 °C and further stirred for 3h at -20 °C. The reaction was quenched by addition of water (7 mL) and allowed to warm up to room temperature. The reaction mixture further stirred for 1h, recooled to 0 °C. To this quenched reaction mixture NaOH (5 mL, 30 % w/v), saturated NaCl solution (6 mL) were added and stirred for 10 min at 0 °C followed by the removal of dichloromethane under reduced pressure. The residue extracted with diethyl ether, combined extracts washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography using gradient elution of 5:1 to 3:1 pentane/ethyl acetate mixture as eluent to afford pure epoxy alcohol **117** as a syrupy liquid.[112]

Yield: 204 mg (0.81 mmol, 70%, 96% *ee*).

Hydrodex-β-6-TBDMS chiral column; Injection mode: split ratio 20:1; Temperature program: 50 °C for 5 min, then with 0.2 °C/min up to 220 °C; R_t: 450.71 min.(minor), 451.59 min.(major).

R_f = 0.30 (pentane/ethyl acetate 1:1).

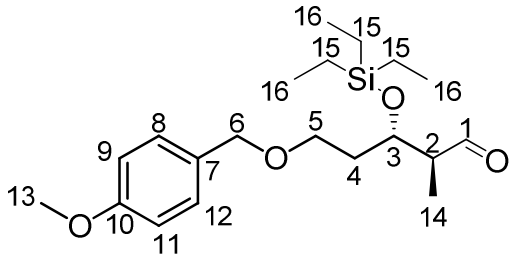
EI-MS (70 eV): *m/z* (%): 252(1) $[M]^+$, 236(1), 218(1), 177(1), 152(2), 147(1), 137(7), 136(7), 135(5), 122(13), 121(100), 107(2), 101(2), 91(5), 89(2), 78(10), 77(9), 65(2), 57(2), 55(2), 52(2), 43(4), 41(4), 39(3).

^{1}H-NMR (600 MHz, CHLOROFORM-*d*) δ [ppm] = 7.23 - 7.27 (2H, m, *J* = 8.7 Hz, H-8, 12), 6.87 - 6.91 (2H, m, *J* = 8.7 Hz, *J* = 2.1 Hz, H-9, 11), 4.47 (2H, q, *J* = 11.5 Hz, H-6), 3.81 (3H, s, H-13), 3.63 (2H, ddd, *J* = 10.5 Hz, *J* = 9.4 Hz, *J* = 3.8 Hz, H-5), 3.30 (1H, d, *J* = 10.2 Hz, -O*H*), 2.79 (1H, dd, *J* = 9.8 Hz, *J* = 4.0 Hz, H-3), 2.09 (1H, dq, *J* = 14.7 Hz, *J* = 7.5 Hz, H-4),

1.71 - 1.79 (1H, m, *J* = 11.5 Hz, *J* = 10.5 Hz, *J* = 9.0 Hz, *J* = 4.1 Hz, *J* = 1.7 Hz, H-4), 1.42 (3H, s, H-14).

^{13}C-NMR (151 MHz, CHLOROFORM-*d*) δ [ppm] = 159.5 (C-10), 129.7 (C-8, 12), 129.0 (C-7), 113.9 (C-9, 11), 73.2 (C-6), 66.2 (C-5), 64.1 (C-1), 62.3 (C-2), 60.4 (C-3), 55.2 (C-13), 29.0 (C-4), 20.4 (C-14).

6.2.54 (2*S*,3*S*)-5-((4-Methoxybenzyl)oxy)-2-methyl-3-((triethylsilyl)oxy)pentanal (119)

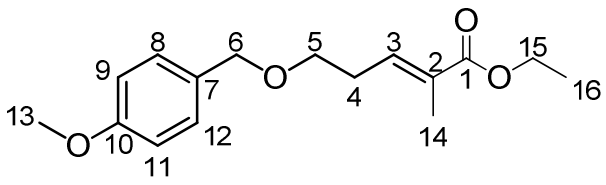


To the suspension of activated powdered 4 A° molecular sieves (100 mg, 49 wt%), epoxy alcohol **117** (202 mg, 0.80 mmol) in anhydrous dichloromethane (3 mL), DIPEA (155 mg, 1.2 mmol) at room temperature under inert atmosphere were added and stirred for 10 minutes. TESOTf **118** (0.26 mL, 1.12 mmol) was added to this reaction mixture at -42 °C, stirred for 90 minutes and poured into diethyl ether (80 mL). The resulting solution washed with water, saturated $CuSO_4$ solution, 5% $NaHCO_3$ solution, dried with $MgSO_4$, filtered, and concentrated in vacuo to obtain the crude aldehyde **119**.[132]

Yield: 267 mg (0.73 mmol, 91%, 75% *d.e.*).

EI-MS (70 eV): *m/z* (%): 337(1) $[M-29]^+$, 332(2), 331(6), 316(1), 315(4), 303(1), 301(2), 219(6), 217(15), 216(18), 215(100), 200(4), 199(24), 190(3), 189(14), 185(3), 173(3), 172(5), 171(26), 161(7), 159(14), 157(5), 147(7), 145(11), 144(5), 143(36), 141(2), 137(5), 133(6), 131(12), 129(7), 127(2), 123(3), 119(7), 117(16), 116(8), 115(67), 113(5), 109(4), 108(7), 105(7), 103(17), 101(8), 99(3), 97(25), 95(4), 94(9), 93(3), 91(5), 89(5), 88(8), 87(70), 86(4), 85(7), 83(27), 80(4), 75(26), 73(7), 71(3), 69(4), 67(4), 63(2), 61(5), 60(5), 59(60), 58(10), 57(10), 55(6), 53(3), 47(18), 45(17), 43(15), 41(10), 40(4), 39(3).

6.2.55 (*E*)-Ethyl 5-((4-methoxybenzyl)oxy)-2-methylpent-2-enoate (124)



To the solution of aldehyde **41** (324 mg, 1.6 mmol) in anhydrous dichloromethane (8 mL) at room temperature under inert atmosphere, stabilized ylide $Ph_3P{=}C(CH_3)CO_2Et$ **123** (1.2 g, 3.3 mmol) was added and stirred for 34 h. The solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography using gradient elution of 10:1 to 2:1 pentane/TBME mixture as eluent to afford the ester **124** as fruity odor yellow oil.[113]

Yield: 418 mg (1.5 mmol, 90%).

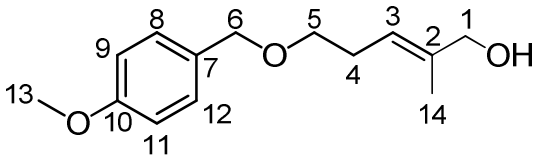
R_f = 0.30 (pentane/TBME 1:1).

EI-MS (70 eV): *m/z* (%): 278(1) $[M]^+$, 260(1), 248(2), 234(1), 233(1), 232(2), 206(1), 205(8), 204(1), 201(2), 187(1), 186(1), 161(2), 159(2), 142(5), 138(2), 137(4), 136(3), 135(5), 122(9), 121(100), 114(3), 113(2), 111(2), 109(2), 107(2), 106(3), 98(3), 97(2), 94(2), 92(2), 91(7), 90(2), 89(4), 84(2), 83(2), 82(3), 79(2), 78(17), 77(15), 71(1), 69(2), 67(4), 65(4), 63(3), 55(5), 54(4), 53(6), 52(4), 51(4), 50(2), 45(1), 43(6), 41(5), 39(7).

^{1}H-NMR (400 MHz, CHLOROFORM-*d*) δ [ppm] = 7.26 (2H, td, *J* = 8.3 Hz, *J* = 1.8 Hz, H-8, 12), 6.88 (2H, td, *J* = 8.8 Hz, *J* = 2.0 Hz, H-9, 11), 6.78 (1H, tq, *J* = 7.0 Hz, *J* = 2.8 Hz, H-3), 4.46 (2H, s, H-6), 4.19 (2H, qq, *J* = 7.0 Hz, *J* = 2.5 Hz, H-15), 3.80 (3H, s, H-13), 3.53 (2H, t, *J* = 6.8 Hz, H-5), 2.48 (2H, dt, *J* = 7.0 Hz, *J* = 0.8 Hz, H-4), 1.84 (3H, dt, *J* = 2.5 Hz, *J* = 0.8 Hz, H-14), 1.29 (3H, td, *J* = 7.0 Hz, *J* = 0.8 Hz, H-16).

^{13}C-NMR (101 MHz, CHLOROFORM-*d*) δ [ppm] = 168.0 (C-1), 159.2 (C-10), 138.3 (C-3), 130.2 (C-7), 129.4 (C-2), 129.2 (C-8, 12), 113.7 (C-9, 11), 72.6 (C-6), 68.3 (C-5), 60.4 (C-15), 55.2 (C-13), 29.4 (C-4), 14.2 (C-16), 12.5 (C-14).

6.2.56 (*E*)-5-((4-Methoxybenzyl)oxy)-2-methylpent-2-en-1-ol (125)



To the solution of ester **124** (390 mg, 1.4 mmol) in anhydrous diethyl ether (5 mL), at 0 °C under inert atmosphere, LAH (106 mg, 2.8 mmol) was added portion wise and stirred for 15 minutes. The reaction mixture was quenched with saturated aqueous Na_2SO_4 solution at 0 °C for 15 minutes. Precipitated solid was filtered through a short pad of celite and the filter cake was washed with diethyl ether. The filtrate and washings were combined and washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography with 4:1 pentane/TBME mixture as eluent to afford the desired *trans*- allylic alcohol **125** exclusively as syrupy liquid.

Yield: 287 mg (1.2 mmol, 87%).

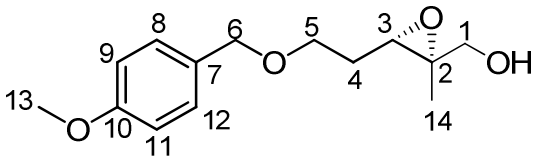
R_f = 0.43 (pentane/ ethyl acetate 1:1).

EI-MS (70 eV): *m/z* (%): 236(1) $[M]^+$, 218(1), 205(1), 143(2), 142(15), 137(3), 136(3), 135(7), 127(1), 122(9), 121(100), 114(5), 113(1), 109(2), 107(2), 106(3), 105(1), 99(4), 94(2), 92(3), 91(9), 90(3), 89(4), 84(3), 83(1), 82(4), 81(1), 79(3), 78(21), 77(18), 71(2), 69(3), 68(1), 67(4), 66(2), 65(5), 64(2), 63(3), 56(1), 55(6), 54(5), 53(8), 52(5), 51(6), 50(2), 45(3), 43(7), 40(2), 39(10).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.15 - 7.19 (2H, m, H-8, 12), 6.77 - 6.82 (2H, m, H-9, 11), 5.33 (1H, qt, *J* = 7.2 Hz, *J* = 2.7 Hz, H-3), 4.36 (2H, s, H-6), 3.88 (2H, s, H-1), 3.71 (3H, s, H-13), 3.37 (2H, t, *J* = 7.0 Hz, H-5), 2.26 (2H, tq, *J* = 7.0 Hz, *J* = 0.9 Hz, H-4), 1.58 (3H, s, H-14).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 159.0 (C-10), 136.6 (C-2), 130.3 (C-7), 129.2 (C-8, 12), 121.7 (C-3), 113.7 (C-9, 11), 72.4 (C-6), 69.3 (C-1), 68.4 (C-5), 55.1 (C-13), 28.2 (C-4), 13.7 (C-14).

6.2.57 ((2*S*,3*S*)-3-(2-((4-Methoxybenzyl)oxy)ethyl)-2-methyloxiran-2-yl)methanol (126)



To a suspension of activated 4 A° molecular sieves (150 mg, 60 wt %) in anhydrous DCM (4 mL) at -30 °C under inert atmosphere Ti(O*i*-Pr)$_4$(313 mg, 1.1 mmol), (+)-DET (0.3 mL, 1.32 mmol) were added and stirred for 30 minutes. Allylic alcohol **125** (250 mg, 1.1 mmol) in anhydrous DCM (4 mL) was added dropwise using a syringe pump over a period of 25 minutes at -35 °C. The reaction mixture stirred for 30 minutes followed by the adition of TBHP (0.6 mL, 3.3 mmol, 5.5 M in nonane) at -35 °C and stirred for 3h at -20 °C. The reaction was quenched with water (7 mL) and allowed to warm up to room temperature and stirred for 1h. The quenched reaction mixture was recooled to 0 °C followed by the addition of NaOH (8 mL, 30 % w/v), saturated NaCl solution (5 mL) and stirred for 10 min at 0 °C followed by the removal of dichloromethane under reduced pressure. The residue extracted with diethyl ether, combined extracts washed with brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography using gradient elution of 4:1 to 2:1 pentane/ethyl acetate mixture as eluent to afford pure epoxy alcohol **126** as a cloudy liquid.[133]

Yield: 180 mg (0.72 mmol, 65%, 98% *ee*).

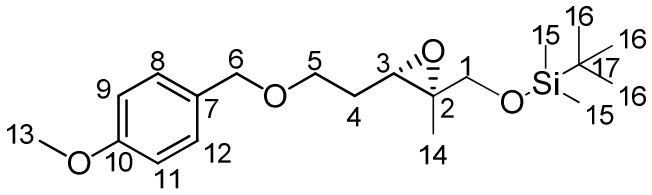
R_f = 0.28 (pentane/ethyl acetate 1:1).

EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 324(1) $[M]^+$, 306(1), 294(1), 278(1), 261(1), 250(1), 233(1), 221(1), 209(1), 203(3), 159(1), 151(2), 145(1), 142(3), 138(2), 137(18), 136(5), 135(6), 131(2), 130(2), 129(3), 122(10), 121(100), 116(1), 115(7), 113(2), 109(3), 107(2), 106(2), 105(2), 103(5), 101(2), 99(2), 98(2), 94(2), 92(2), 91(6), 90(2), 89(3), 85(4), 83(2), 82(3), 79(2),78(12), 77(12), 76(1), 75(13), 74(3), 73(25), 69(1), 67(3), 66(1), 65(3), 63(2), 61(2), 59(6), 58(2), 57(3), 55(3), 53(1), 52(2), 51(2), 47(2), 45(5), 43(5), 41(3), 39(3).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.15 - 7.23 (2H, m, *J* = 8.7 Hz, H-8, 12), 6.76 - 6.85 (2H, m, *J* = 8.7 Hz, *J* = 2.1 Hz, H-9, 11), 4.39 (2H, s, H-6), 3.73 (3H, s, H-13), 3.54 (2H, t, *J* = 6.8 Hz, H-5), 3.43 - 3.63 (2H, m, *J* = 12.1 Hz, *J* = 3.2 Hz, *J* = 1.5 Hz, H-1), 3.10 (1H, dd, *J* = 7.0 Hz, *J* = 5.3 Hz, H-3), 1.68 - 1.93 (2H, m, *J* = 14.2 Hz, *J* = 7.4 Hz, *J* = 1.9 Hz, H-4), 1.21 (3H, s, H-14).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 159.2 (C-10), 130.3 (C-7), 129.2 (C-8, 12), 113.8 (C-9, 11), 72.7 (C-6), 67.0 (C-5), 65.4 (C-1), 60.8 (C-2), 57.9 (C-3), 55.2 (C-13), 28.9 (C-4), 14.3 (C-14).

6.2.58 *tert*-Butyl(((2S,3S)-3-(2-((4-methoxybenzyl)oxy)ethyl)-2-methyloxiran-2-yl)methoxy)dimethylsilane (130)



To the solution of epoxy alcohol **126** (150 mg, 0.6 mmol) in anhydrous dichloromethane (2 mL) at room temperature under inert atmosphere, imidazole (45 mg, 0.66 mmol), *tert*-butyldimethylsilyl chloride **129** (199 mg, 1.32 mmol) were added successively and stirred for 1 h. The reaction mixture diluted with dichloromethane, washed with water, saturated brine, dried with Na_2SO_4, filtered, and concentrated in vacuo. Purification of the crude product by flash column chromatography using gradient elution of 10:1 to 5:1 pentane/ TBME mixture as eluent afforded the TBDMS protected epoxy alcohol **130** as colorless liquid.[114]

Yield: 215 mg (0.58 mmol, 98%, 98% *d.e.*).

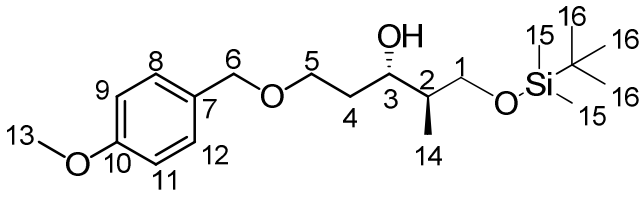
R_f = 0.68 (pentane/TBME 1:1).

EI-MS (70 eV): *m/z* (%): 366(1) $[M]^+$, 350(1), 221(2), 1773(2), 171(2), 158(3), 143(2), 135(4), 130(9), 129(3), 122(11), 121(100), 115(3), 113(3), 109(1), 107(3), 103(1), 101(4), 97(2), 91(4), 89(4), 85(3), 78(8), 77(5), 75(8), 73(8), 65(2), 59(4), 58(3), 57(4), 51(3).

^{1}H-NMR (300 MHz, CHLOROFORM-*d*) δ [ppm] = 7.20 (2H, ddt, *J* = 8.7 Hz, *J* = 3.8 Hz, *J* = 2.1 Hz, H-8, 12), 6.81 (2H, dt, *J* = 8.7 Hz, *J* = 2.1 Hz, H-9, 11), 4.40 (2H, s, H-6), 3.74 (3H, s, H-13), 3.55 (2H, dd, *J* = 5.9 Hz, *J* = 1.1 Hz, H-1), 3.52 (2H, t, *J* = 2.5 Hz, H-5), 2.95 (1H, dd, *J* = 7.2 Hz, *J* = 5.3 Hz, H-3), 1.85 (1H, ddt, *J* = 14.0 Hz, *J* = 7.2 Hz, *J* = 5.3 Hz, H-4), 1.73 (1H, ddt, *J* = 12.9 Hz, *J* = 7.0 Hz, *J* = 5.7 Hz, H-4), 1.21 (3H, s, H-14), 0.84 (9H, s, H-16), 0.04 (6H, s, H-15).

^{13}C-NMR (75 MHz, CHLOROFORM-*d*) δ [ppm] = 159.2 (C-10), 130.4 (C-7), 129.2 (C-8, 12), 113.8 (C-9, 11), 72.8 (C-6), 67.8 (C-5), 67.3 (C-1), 60.9 (C-2), 58.4 (C-3), 55.3 (C-13), 29.2 (C-4), 25.9 (C-16), 18.3 (C-17), 14.2 (C-14), -5.21 (C-15).

6.2.59 (2*R*,3*S*)-1-((*tert*-Butyldimethylsilyl)oxy)-5-((4-methoxybenzyl)oxy)-2-methylpentan-3-ol (131)



To the mixture of $Pd_2(dba)_3CHCl_3$ **127** (7 mg, 5 mol%) and n-Bu_3P (4 µL) in dioxane (244 µL) a mixture of HCOOH (25 µL) and Et_3N (36 µL, 0.26 mmol) in dioxane (156 µL) at room temperature was added. The mixture was stirred for 15 minutes. The oxirane **130** (50 mg, 0.13 mmol) in dioxane (330 µL) was added dropwise and monitored by TLC until the consumption of the oxirane **130**. The reaction quenched with water and the aqueous layer was extracted with diethyl ether. The combined organic layers washed with saturated NH_4Cl solution, brine dried with $MgSO_4$, filtered, and concentrated in vacuo to afford the **131** as crude product.[115]

Yield: 43 mg (0.12 mmol, 87%, 90% *d.e.*).

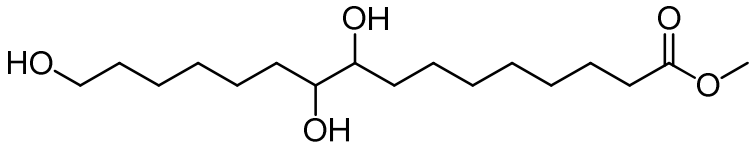
R_f = 0.34 (pentane/ethyl acetate 1:1).

EI-MS (70 eV) (MSTFA derivative): *m/z* (%): 440(1) $[M]^+$, 383(1), 360(1), 345(1), 329(1), 309(1), 292(1), 279(1), 264(1), 221(1), 214(1), 201(1), 194(1), 171(1), 159(1), 143(3), 137(5), 135(3), 131(4), 126(1), 122(10), 121(100), 115(9), 101(3), 99(2), 91(4), 89(3), 85(4), 78(8), 77(9), 75(16), 73(8), 65(2), 59(5), 57(9), 55(4), 51(2), 45(4), 43(4), 41(9), 39(6).

^{1}H-NMR (600 MHz, CHLOROFORM-*d*) δ [ppm] = 7.22 (2H, ddd, *J* = 8.7 Hz, *J* = 4.9 Hz, *J* = 2.8 Hz, H-8, 12), 6.83 (2H, ddt, *J* = 8.7 Hz, *J* = 3.2 Hz, *J* = 2.1 Hz, H-9, 11), 4.41 (2H, d, *J* = 1.9 Hz, H-6), 3.78 (0H, td, *J* = 4.3 Hz, *J* = 1.9 Hz, H-3), 3.76 (3H, s, H-13), 3.57 (1H, d, *J* = 1.3 Hz, H-1), 3.55 (2H, dd, *J* = 4.0 Hz, *J* = 2.4 Hz, H-5), 3.51 (1H, dd, *J* = 11.3 Hz, *J* = 3.8 Hz, H-1), 1.87 (1H, dtd, *J* = 12.0 Hz, *J* = 7.5 Hz, *J* = 2.3 Hz, H-2), 1.76 (1H, dddt, *J* = 14.3 Hz, *J* = 10.5 Hz, *J* = 4.7 Hz, *J* = 1.3 Hz, H-4), 1.69 (1H, td, *J* = 12.0 Hz, *J* = 4.7 Hz, H-4), 0.85 - 0.94 (9H, s, H-16), 0.84 (3H, d, *J* = 6.8 Hz, H-14), 0.04 (6H, s, H-15).

^{13}C-NMR (151 MHz, CHLOROFORM-*d*) δ [ppm] = 159.1 (C-10), 130.3 (C-7), 129.2 (C-8, 12), 113.7 (C-9, 11), 72.8 (C-6), 72.7 (C-3), 67.6 (C-5), 67.0 (C-1), 55.2 (C-13), 38.5 (C-2), 34.7 (C-4), 25.8 (C-16), 18.3 (C-17), 13.5 (C-14), -5.4 (C-15).

6.2.60 Methyl 9,10,16-trihydroxyhexadecanoate (137)



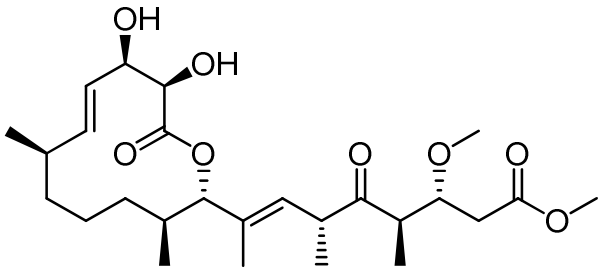
Synthesis of diazomethane:

Diazomethane was generated by addition of potassium hydroxide solution (1.08 M in methanol-H2O [1:1, 4 ml]) to a stirred Diazald® solution (0.52 M diethyl ether-diethyleneglycol monoethyl ether [1:1, 4ml]) in a screw-cap glass vial with polytetrafluoroethylene (PTFE) tubing attached. The diazomethane evolved, which is yellow, was allowed to pass through a trap to avoid addition of by-products and then further into the glass vial containing the reaction mixture.[116]

To aleuritic acid **135** (1 mg, 3.3 µmol) in MeOH and diethyl ether (1:1, 1 mL), Diazomethane **136** gas was bubbled through the solution in excess while stirring at 0 °C for 1 h. The excess of diazomethane and solvent were removed under the stream of Nitrogen and the crude methyl ester **137** was analyzed by HPLC/MS.

ESI-HRMS: $[M+H]^+$ 319.20, $[M+Na-H]^+$ 341.32, $[2M-2H]^+$ 636.64.

6.2.61 (3*R*,4*R*,6*R*,*E*)-Methyl-8-((2*S*,3*S*,7*R*,10*R*,11*R*,*E*)-10,11-dihydroxy-3,7-dimethyl-12-oxooxacyclododec-8-en-2-yl)-3-methoxy-4,6-dimethyl-5-oxonon-7-enoate (138)



To carolacton **13** (1 mg, 2.13 µmol) in MeOH and diethyl ether (1:1, 1mL), Diazomethane **136** gas was bubbled through the solution in excess while stirring at 0 °C for 1 h. The excess of diazomethane and solvent were removed under the stream of Nitrogen to obtain methyl ester **138** of carolacton and analyzed by HPLC/MS.[43]

ESI-HRMS: $[M+H]^+$ 483.28, $[M+Na]^+$ 505.40, $[2M+Na-H]^+$ 986.52.

8 Literature

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8 Abbreviations

α	Alpha
[α]	Optical rotation
br. s.	broad signal
β	Beta
CDA	Chiral derivatizing agent
CoA	Coenzyme A
δ	Chemical Shift (in ppm)
d	Doublet
d.e.	Diastereomeric excess
d.r.	Diastereomeric ratio
ds	Diastereomer
ee.	Enantiomeric excess
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DET	Diethyl Tartarate
DIBAL-H	Diisobutylaluminium hydride
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EI	Electron impact
EtMgBr	Ethylmagnesium bromide
eV	Electron volt
γ	Gamma
GC	Gas chromatography
GC/MS	Gas chromatography coupled with Mass spectrometry
HMPA	Hexamethylphosphoramide
HPLC	High performance liquid chromatography
HPLC/MS	High performance liquid chromatography coupled with mass spectrometry
IR	Infrared (Spectroscopy)
KHMDS	Potassium hexamethyldisilazane
KO*t*Bu	Potassium *tert*-butoxide
J	Coupling constant

LAH	Lithiumaluminiumhydride
LDA	Lithium diisopropylamide
LiHMDS	Lithium hexamethyldisilazane
m	Multiplet
MBp	Million base pairs
MSTFA	(*N*-methyl-*N*-trimethylsilyltrifluoroacetamide)
MPA	α-methoxy-α- phenylacetic acid
MTPA	α-methoxy-α-trifluoromethyl-α-phenylacetic acid
m/z	Mass-to-charge ratio
MS	Mass spectrometry
n-BuLi	n-Butyllithium
NaHMDS	Sodium hexamethyldisilazane
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser Enhancement Spectroscopy
PivCl	Pivaloylchloride
PMB	*para*-Methoxybenzyl
q	Quartet
quin	Quintet
R_f	Retention factor (Ratio of fronts)
rt	room temperature
s	Singlet
S.mutans	*Streptococcus mutans*
t	Triplet
TBDMS	*tert*-Butyldimethylsilyl
TBHP	*tert*-Butyl hydroperoxide
TBME	*tert*-Butylmethylether
TES	Triethylsilyl
TESOTf	Triethylsilyltriflate
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin layer chromatography
TMS	Trimethylsilyl

9 Appendix

9.1 Mass spectra

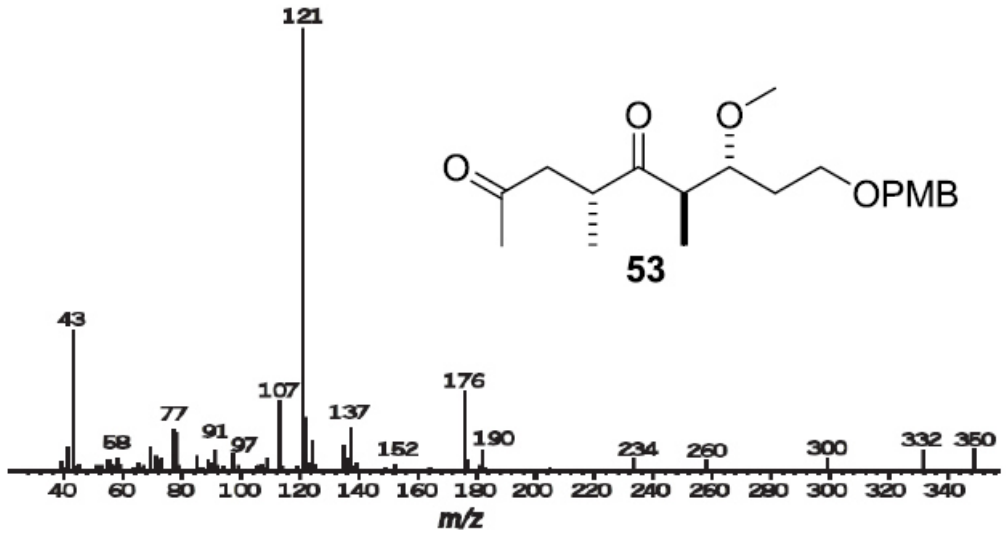

Figure 80: Mass spectrum of (4*RS*,6*RS*,7*RS*)-7-Methoxy-9-((4-methoxybenzyl)oxy)-4,6-dimethylnonane-2,5-dione (**53**)

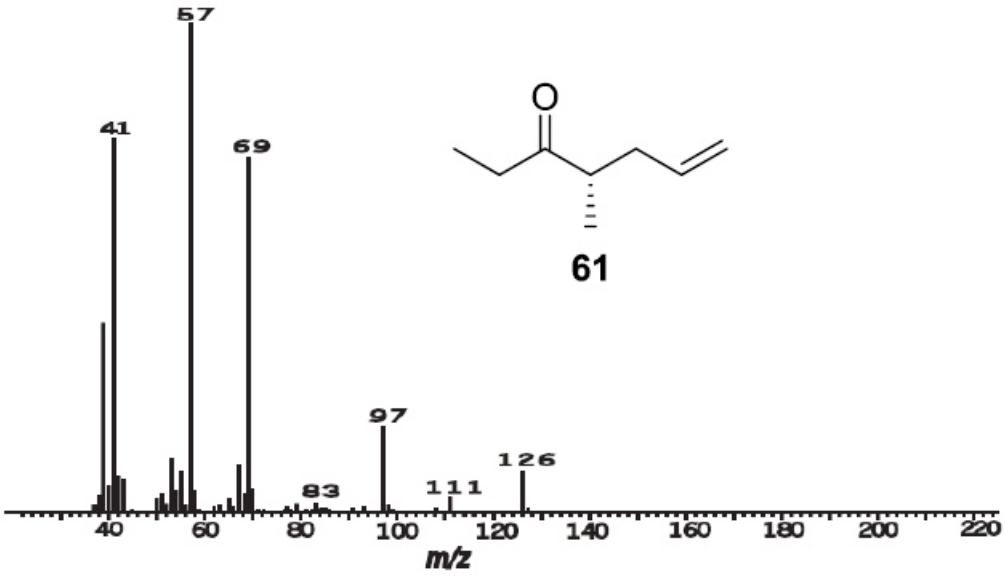

Figure 81: Mass spectrum of (R)-4-Methylhept-6-en-3-one (**61**)

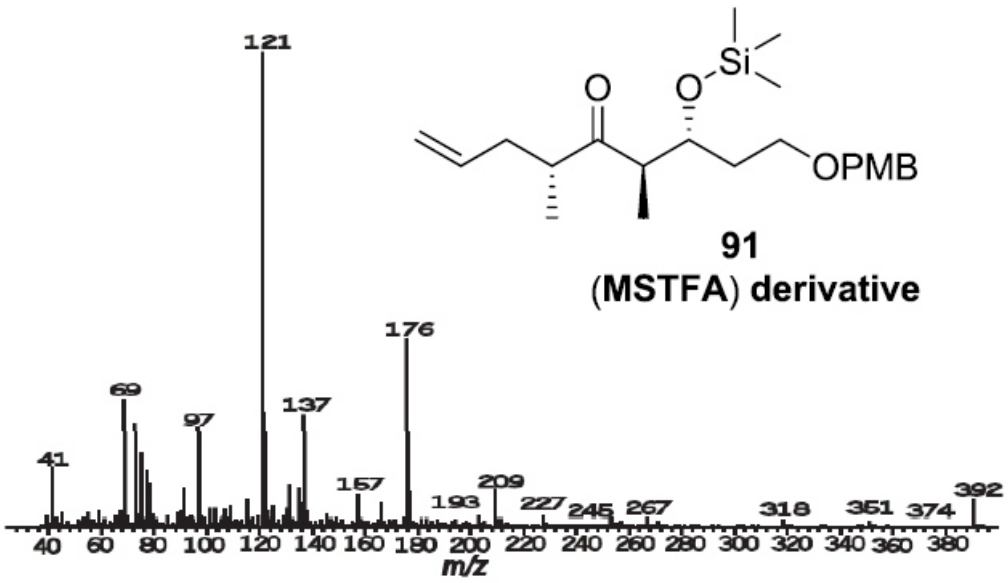

Figure 82: Mass spectrum of corresponding MSTFA derivative of (4*S*,6*S*,7*S*)-7-Hydroxy-9-((4-methoxybenzyl)oxy)-4,6-dimethylnon-1-en-5-one (**91**)

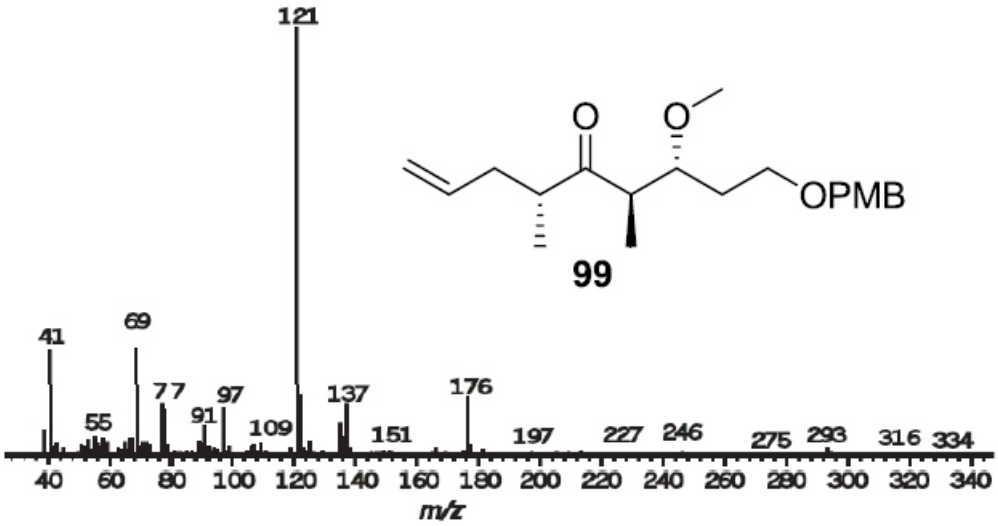

Figure 83: Mass spectrum of (4*S*,6*S*,7*S*)-7-methoxy-9-((4-methoxybenzyl)oxy)-4,6-dimethylnon-1-en-5-one (**99**)

Acknowledgements

I owe my deepest gratitude to Prof. Dr. Stefan Schulz who was an excellent supervisor to work with, for giving me an opportunity to work in his research group, for his patience, motivation and enthusiasm throughout my research work.

I am also greatly indebted to my cooperation partners Prof. Dr. I. Wagner-Döbler and the team of workgroups in the BIOINSYS project who in some way contributed to the progress and publication of the part of the work contained herein.

Besides my mentor and cooperation partners, I would like to express sincere regards to the rest of my thesis committee: Prof. Dr. Thomas Lindel for being both as a second referee and examiner and Prof. Ulrich Engelhardt for being the third examiner for my doctoral defense.

I also thank all my colleagues from our research group: Dr. Insa Bergmann, Hilke Bruns, Jana Caspers, Dr. Satya Chinta, Dr. Jeroen Dickschat, Dr. Christopher Häberlein, Tim Gauger, Ulrike Groenhagen, Susann Hötling, Elena Jerhot, Julia Kiefer, Michael Kostka, Michael Maczka, Florian Mann, Jasmin Müller, Dr. Thorben Nawrath, Dr. Alexander Neumann, Pardha Saradhi Peram, Dr. Dennis Poth, Srinivasa Rao Ravella, René Röpke, Anna-Lena von Rymon-Lipinski, Andreas Schmidt, Witali Schmidt, Dr. Thies Marten Schulze, Martin Stürminger, Dr. Selma Yildizhan and Lisa Ziesche for their discussion, support and friendly help.

I thank the NMR department and all of the staff in the chemical stores as well as all the graduate trainees who helped me during my project especially with the large column purifications.

I am indebted to my loving parents Satyavathi Surapaneni and Venkata Ratnam Surapaneni for their continous support all the way through my career and my wife Shanti Surapaneni for her unending support in all my works.